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(54) Title: ACRP30-LIKE POLYNUCLEOTIDES, POLYPEPTIDES, AND ANTIBODIES

(57) Abstract: The present invention relates to novel human ACRP30-Like polypeptides and isolated nucleic acids containing the coding regions of the genes encoding such polypeptides. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human ACRP30-Like polypeptides. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human ACRP30-Like polypeptides.

ACRP30-LIKE POLYNUCLEOTIDES, POLYPEPTIDES, AND ANTIBODIES

FIELD OF THE INVENTION

[0001] The present invention relates to novel ACRP30-Like proteins. More specifically, isolated nucleic acid molecules are provided encoding novel ACRP30-Like polypeptides. Novel ACRP30-Like polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human ACRP30-Like polynucleotides and/or polypeptides. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel ACRP30-Like polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and function of the polypeptides of the present invention.

BACKGROUND OF INVENTION

[0002] Over the past few decades, an increasing percentage of the population has become diabetic. Diabetes mellitus is categorized into two types: Type I Insulin-Dependent Diabetes Mellitus (IDDM) or Type II Non-Insulin-Dependent Diabetes Mellitus (NIDDM). Type I IDDM is an autoimmune disorder in which the insulin-secreting pancreatic beta cells are destroyed. In these individuals, recombinant insulin therapy is employed to maintain glucose homeostasis and normal energy metabolism. Type II NIDDM, on the other hand, is a polygenic disorder with no one gene responsible for the progression of the disease.

[0003] In NIDDM, insulin resistance eventually leads to the abolishment of insulin secretion resulting in insulin deficiency. Insulin resistance, at least in part, ensues from a block at the level of glucose uptake and phosphorylation in humans. Diabetics demonstrate a decrease in expression in adipose tissue of insulin-receptor substrate 1 ("IRS1"), glucose transporter 4 ("GLUT4") , and the novel abundant protein M gene transcript 1 ("apM1"), as well as other as of yet unidentified factors.

[0004] Insulin action in adipose and muscle tissues is mediated by the interaction of insulin with its receptor, subsequent phosphorylation events, protein-protein interactions, activation of phosphoinositide-3-kinase, downstream activation of protein kinase B (also known as Akt) and protein kinase C isoforms, and, ultimately, translocation of GLUT4 from a specialized intracellular compartment to the plasma membrane, allowing for glucose uptake. While the mechanism(s) of diabetes have been determined to some extent, all the genetic factors involved need to be elucidated.

[0005] Insulin affects fat, muscle, and liver. In fat, glucose is converted to alpha-glycerophosphate which then esterifies the free fatty acids to be used in triglyceride synthesis and storage. In muscle and liver, glucose is phosphorylated and directed for glycogen synthesis and storage. Thus, insulin plays a major role in energy homeostasis.

[0006] Insulin promotes GLUT4 translocation and adipocyte complement related protein 30 ("ACRP30") secretion. ACRP30 or AdipoQ is the mouse ortholog of human apM1 also known as gelatin binding protein 28 kDa ("GBP28") and adiponectin. ACRP30 acts in a similar manner to insulin insofar as lowering blood glucose levels and lowering elevated levels of plasma free fatty acids. ACRP30 lowers blood glucose levels by enhancing the effect of insulin on hepatic glucose production, and lowers levels of plasma free fatty acids by increasing muscle fatty acid oxidation. Low levels of plasma ACRP30 are associated with, and related to, the degree of insulin resistance and hyperinsulinemia.

[0007] ACRP30 is homologous to complement factor C1q, hibernation-specific proteins HP-20, -25 & -27, and cerebellin in its primary sequence. It has an amino-terminal signal sequence, a stretch of amino acids with no known homology, "Gly-X-Y" collagen-like repeats (where X and Y can be any amino acid), and a carboxy-terminal globular domain. The globular domain is similar in crystal structure to C1q and tumor necrosis factor alpha ("TNFα"). ACRP30 is specifically expressed in adipocytes over 100 fold during adipogenesis. TNFα has been implicated in insulin resistance in obesity and NIDDM. Due to their structural similarities, ACRP30 may act as an antagonist to TNFα and ameliorate and/or reverse insulin resistance. ACRP30 may also have immune-related functions in addition to its potential involvement in obesity and diabetes mellitus.

[0008] Insulin is the major regulator of energy metabolism. Malfunctioning of any step(s) in insulin secretion and/or action can lead to many disorders, for example the

dysregulation of oxygen utilization, adipogenesis, glycogenesis, lipogenesis, glucose uptake, protein synthesis , thermogenesis, and maintenance of the basal metabolic rate. Said malfunctioning is detrimental, and results in diseases and/or disorders that include, but are not limited to, hyperinsulinemia, insulin resistance, insulin deficiency, hyperglycemia, hyperlipidemia, hyperketonemia, and diabetes.

[0009] Secondary effects can also be debilitating. They are numerous and include, but are not limited to, obesity, forms of blindness (cataracts and diabetic retinopathy), limb amputations, kidney failure, fatty liver, and coronary artery disease.

[0010] Current drugs used to treat insulin resistance and/or diabetes (e.g. insulin secratogogues – sulfonylurea, insulin sensitizers – thiazolidenediones and metformin, and alpha-glucosidase and lipase inhibitors) are inadequate. Accordingly, polynucleotides, polypeptides, and antibodies corresponding to ACRP30 or related homologs have utilities that include, but are not limited to, the prognosis, diagnosis, and/or treatment of insulin resistance and diabetes mellitus Types I and II in lean and obese patients.

SUMMARY OF THE INVENTION

[0011] The present invention includes isolated nucleic acid molecules comprising, or alternatively, consisting of a polynucleotide sequence disclosed in the sequence listing and/or contained in a human cDNA plasmid described in Table 1 and deposited with the American Type Culture Collection (ATCC). Fragments, variants, and derivatives of these nucleic acid molecules are also encompassed by the invention. The present invention also includes isolated nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide encoding ACRP30-Like polypeptides. The present invention further includes ACRP30-Like polypeptides encoded by these polynucleotides. Further provided for are amino acid sequences comprising, or alternatively, consisting of, ACRP30-Like polypeptides as disclosed in the sequence listing and/or encoded by the human cDNA plasmids described in Table 1 and deposited with the ATCC. Antibodies that bind these polypeptides are also encompassed by the invention. Polypeptide fragments, variants, and derivatives of these amino acid sequences are also encompassed by the invention, as are polynucleotides encoding these polypeptides and antibodies that bind these polypeptides.

DETAILED DESCRIPTION

Tables

[0012] Table 1 summarizes ATCC Deposits, Deposit dates, and ATCC designation numbers of deposits made with the ATCC in connection with the present application. Table 1 further summarizes the information pertaining to each "Gene No." described below, including cDNA plasmid identifier, the type of vector contained in the cDNA plasmid identifier, the nucleotide sequence identifier number, nucleotides contained in the disclosed sequence, the location of the 5' nucleotide of the start codon of the disclosed sequence, the amino acid sequence identifier number, and the last amino acid of the ORF encoded by the disclosed sequence.

[0013] Table 2 indicates public ESTs, of which at least one, two, three, four, five, ten, or more of any one or more of these public EST sequences are optionally excluded from certain embodiments of the invention.

[0014] Table 3 summarizes the expression profile of polynucleotides corresponding to the clones disclosed in Table 1. The first column provides a unique clone identifier, "cDNA Plasmid:V", for a cDNA clone related to each contig sequence disclosed in Table 1. Column 2, "Library Code" shows the expression profile of tissue and/or cell line libraries which express the polynucleotides of the invention. Each Library Code in column 2 represents a tissue/cell source identifier code corresponding to the Library Code and Library description provided in Table 5. Expression of these polynucleotides was not observed in the other tissues and/or cell libraries tested. One of skill in the art could routinely use this information to identify tissues which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue expression.

[0015] Table 4, column 1, provides a nucleotide sequence identifier, "SEQ ID NO:X," that matches a nucleotide SEQ ID NO:X disclosed in Table 1, column 5. Table 4, column 2, provides the chromosomal location, "Cytologic Band or Chromosome," of polynucleotides corresponding to SEQ ID NO:X. Chromosomal location was determined by finding exact matches to EST and cDNA sequences contained in the NCBI (National Center for Biotechnology Information) UniGene database. Given a presumptive

chromosomal location, disease locus association was determined by comparison with the Morbid Map, derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD) 2000. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/). If the putative chromosomal location of the Query overlapped with the chromosomal location of a Morbid Map entry, the OMIM reference identification number of the morbid map entry is provided in Table 4, column 3, labelled "OMIM ID." A key to the OMIM reference identification numbers is provided in Table 6.

[0016] Table 5, column 1, provides the Library Code disclosed in Table 3, column 2. Column 2 provides a description of the tissue or cell source from which the corresponding library was derived. Library codes corresponding to diseased tissues are indicated in column 3 with the word "disease". The use of the word "disease" in column 3 is non-limiting. The tissue source of the library may be specific (e.g., a neoplasm), or may be disease-associated (e.g., a tissue sample from a normal portion of a diseased organ). Furthermore, libraries lacking the "disease" designation may still be derived from sources directly or indirectly involved in a disease state or disorder, and therefore may have a further utility in that disease state or disorder.

[0017] Table 6 provides a key to the OMIM reference identification numbers disclosed in Table 4, column 3. OMIM reference identification numbers (Column 1) were derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine, (Bethesda, MD) 2000. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/). Column 2 provides diseases associated with the cytologic band disclosed in Table 4, column 2, as determined from the Morbid Map database.

Definitions

[0018] The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

[0019] In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

[0020] As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X (as described in column 5 of Table 1), or cDNA plasmid:V (as described in column 2 of Table 1 and contained within a pool of plasmids deposited with the ATCC in ATCC Deposit No:Z). For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without a natural or artificial signal sequence, the protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having an amino acid sequence encoded by a polynucleotide of the invention as broadly defined (obviously excluding poly-Phenylalanine or poly-Lysine peptide sequences which result from translation of a polyA tail of a sequence corresponding to a cDNA).

[0021] In the present invention, a representative plasmid containing the sequence of SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC") and/or described in Table 1. As shown in Table 1, each plasmid is identified by a cDNA Plasmid Identifier and the ATCC Deposit Number (ATCC Deposit No:Z). Plasmids that were pooled and deposited as a single deposit have the same ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

[0022] A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, or the complement thereof (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein) and/or sequences contained in cDNA plasmid:V (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein). "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

[0023] Also included within "polynucleotides" of the present invention are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 μg/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

[0024] Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

[0025] Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid

molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

[0026] The polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0027] In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

[0028] "SEQ ID NO:X" refers to a polynucleotide sequence described in column 5 of Table 1, while "SEQ ID NO:Y" refers to a polypeptide sequence described in column 10 of Table 1. SEQ ID NO:X is identified by an integer specified in column 6 of Table 1. The polypeptide sequence SEQ ID NO:Y is a translated open reading frame (ORF) encoded by polynucleotide SEQ ID NO:X. The polynucleotide sequences are shown in the sequence listing immediately followed by all of the polypeptide sequences. Thus, a polypeptide sequence corresponding to polynucleotide sequence SEQ ID NO:2 is the first polypeptide

sequence shown in the sequence listing. The second polypeptide sequence corresponds to the polynucleotide sequence shown as SEQ ID NO:3, and so on.

[0029] The polypeptides of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992)).

[0030] The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced

polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

[0031] The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

[0032] The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the polypeptides of the present invention in methods which are well known in the art.

[0033] By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

[0034] "A polypeptide having functional activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular assay, such as, for example, a biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more

than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

[0035] The functional activity of the polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

[0036] For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the present invention for binding to an antibody to the full length polypeptide, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[0037] In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment, physiological correlates polypeptide of the present invention binding to its substrates (signal transduction) can be assayed.

[0038] In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and fragments, variants derivatives and analogs thereof to elicit polypeptide related biological activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

Polynucleotides and Polypeptides of the Invention

FEATURES OF PROTEIN ENCODED BY GENE NO.: 1

[0039] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0040] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression

of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0041] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 50 as residues: Pro-94 to Lys-102, Phe-130 to Trp-136, Ser-172 to Asn-180, Phe-182 to Phe-189, Pro-191 to Phe-196, and Lys-198 to Glu-228. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0042] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 50 as residues Asp-92 to Leu-229. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0043] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 50 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0044] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0045] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0046] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the

invention shown as SEQ ID NO: 50: G-93 to L-229; P-94 to L-229; C-95 to L-229; P-96 to L-229; Q-97 to L-229; D-98 to L-229; E-99 to L-229; K-100 to L-229; L-101 to L-229; K-102 to L-229; D-103 to L-229; A-104 to L-229; F-105 to L-229; S-106 to L-229; H-107 to L-229; V-108 to L-229; V-109 to L-229; E-110 to L-229; N-111 to L-229; T-112 to L-229; A-113 to L-229; F-114 to L-229; F-115 to L-229; G-116 to L-229; D-117 to L-229; V-118 to L-229; V-119 to L-229; L-120 to L-229; R-121 to L-229; F-122 to L-229; P-123 to L-229; R-124 to L-229; I-125 to L-229; V-126 to L-229; H-127 to L-229; Y-128 to L-229; Y-129 to L-229; F-130 to L-229; D-131 to L-229; H-132 to L-229; N-133 to L-229; S-134 to L-229; N-135 to L-229; W-136 to L-229; N-137 to L-229; L-138 to L-229; L-139 to L-229; I-140 to L-229; R-141 to L-229; W-142 to L-229; G-143 to L-229; I-144 to L-229; S-145 to L-229; F-146 to L-229; C-147 to L-229; N-148 to L-229; Q-149 to L-229; T-150 to L-229; G-151 to L-229; V-152 to L-229; F-153 to L-229; N-154 to L-229; Q-155 to L-229; G-156 to L-229; P-157 to L-229; H-158 to L-229; S-159 to L-229; P-160 to L-229; I-161 to L-229; L-162 to L-229; S-163 to L-229; L-164 to L-229; M-165 to L-229; A-166 to L-229; Q-167 to L-229; E-168 to L-229; L-169 to L-229; G-170 to L-229; I-171 to L-229; S-172 to L-229; E-173 to L-229; K-174 to L-229; D-175 to L-229; S-176 to L-229; N-177 to L-229; F-178 to L-229; Q-179 to L-229; N-180 to L-229; P-181 to L-229; F-182 to L-229; K-183 to L-229; I-184 to L-229; D-185 to L-229; R-186 to L-229; T-187 to L-229; E-188 to L-229; F-189 to L-229; I-190 to L-229; P-191 to L-229; S-192 to L-229; T-193 to L-229; D-194 to L-229; P-195 to L-229; F-196 to L-229; Q-197 to L-229; K-198 to L-229; A-199 to L-229; L-200 to L-229; R-201 to L-229; E-202 to L-229; E-203 to L-229; E-204 to L-229; K-205 to L-229; R-206 to L-229; R-207 to L-229; K-208 to L-229; K-209 to L-229; E-210 to L-229; E-211 to L-229; K-212 to L-229; R-213 to L-229; K-214 to L-229; E-215 to L-229; I-216 to L-229; R-217 to L-229; K-218 to L-229; G-219 to L-229; P-220 to L-229; R-221 to L-229; I-222 to L-229; S-223 to L-229; and R-224 to L-229 of SEQ ID NO: 50.

[0047] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the

complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0048] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 50: D-92 to E-228; D-92 to S-227; D-92 to Q-226; D-92 to S-225; D-92 to R-224; D-92 to S-223; D-92 to I-222; D-92 to R-221; D-92 to P-220; D-92 to G-219; D-92 to K-218; D-92 to R-217; D-92 to I-216; D-92 to E-215; D-92 to K-214; D-92 to R-213; D-92 to K-212; D-92 to E-211; D-92 to E-210; D-92 to K-209; D-92 to K-208; D-92 to R-207; D-92 to R-206; D-92 to K-205; D-92 to E-204; D-92 to E-203; D-92 to E-202; D-92 to R-201; D-92 to L-200; D-92 to A-199; D-92 to K-198; D-92 to Q-197; D-92 to F-196; D-92 to P-195; D-92 to D-194; D-92 to T-193; D-92 to S-192; D-92 to P-191; D-92 to I-190; D-92 to F-189; D-92 to E-188; D-92 to T-187; D-92 to R-186; D-92 to D-185; D-92 to I-184; D-92 to K-183; D-92 to F-182; D-92 to P-181; D-92 to N-180; D-92 to Q-179; D-92 to F-178; D-92 to N-177; D-92 to S-176; D-92 to D-175; D-92 to K-174; D-92 to E-173; D-92 to S-172; D-92 to I-171; D-92 to G-170; D-92 to L-169; D-92 to E-168; D-92 to Q-167; D-92 to A-166; D-92 to M-165; D-92 to L-164; D-92 to S-163; D-92 to L-162; D-92 to I-161; D-92 to P-160; D-92 to S-159; D-92 to H-158; D-92 to P-157; D-92 to G-156; D-92 to Q-155; D-92 to N-154; D-92 to F-153; D-92 to V-152; D-92 to G-151; D-92 to T-150; D-92 to Q-149; D-92 to N-148; D-92 to C-147; D-92 to F-146; D-92 to S-145; D-92 to I-144; D-92 to G-143; D-92 to W-142; D-92 to R-141; D-92 to I-140; D-92 to L-139; D-92 to L-138; D-92 to N-137; D-92 to W-136; D-92 to N-135; D-92 to S-134; D-92 to N-133; D-92 to H-132; D-92 to D-131; D-92 to F-130; D-92 to Y-129; D-92 to Y-128; D-92 to H-127; D-92 to V-126; D-92 to I-125; D-92 to R-124; D-92 to P-123; D-92 to F-122; D-92 to R-121; D-92 to L-120; D-92 to V-119; D-92 to V-118; D-92 to D-117; D-92 to G-116; D-92 to F-115; D-92 to F-114; D-92 to A-113; D-92 to T-112; D-92 to N-111; D-92 to E-110; D-92 to V-109; D-92 to V-108; D-92 to H-107; D-92 to S-106; D-92 to F-105; D-92 to A-104; D-92 to D-103; D-92 to K-102; D-92 to L-101; D-92 to K-100; D-92 to E-99; and D-92 to D-98 of SEQ ID NO: 50.

[0049] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0050] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 50, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0051] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides,

such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0052] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-844, where this portion excludes any integer of amino acid residues from 1 to about 223 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-844, or any integer of amino acid residues from 1 to about 223 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-844. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0053] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0054] Translation products of this gene stimulate glucose transport in adipocytes.

[0055] It has been discovered that this gene is strongly expressed in muscle tissue, and to a lesser extent in neutrophils and lung tissue.

[0056] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, and immunological disorders.

[0057] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., muscle, adipose, immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual

having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0058] The expression of this gene in muscle tissue and structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0059] In addition, expression of this gene in neutrophils and lung tissue and the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are

useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0060] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES FOR PROTEIN ENCODED BY GENE NO.: 2

[0061] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329840), and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543, AAB06706, and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0062] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0063] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 51 as residues: Phe-30 to Cys-37, Arg-91 to Gly-98, Pro-170 to Ala-177, Pro-183 to Gly-193, Pro-206 to Gly-235, Pro-243 to Pro-260, Phe-283 to Gly-311. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0064] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 51 as residues Arg-246 to Pro-421. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0065] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 51 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0066] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0067] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to

induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0068] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 51: G-247 to P-421; P-248 to P-421; P-249 to P-421; G-250 to P-421; P-251 to P-421; P-252 to P-421; G-253 to P-421; P-254 to P-421; P-255 to P-421; G-256 to P-421; P-257 to P-421; P-258 to P-421; G-259 to P-421; P-260 to P-421; P-261 to P-421; A-262 to P-421; P-263 to P-421; V-264 to P-421; G-265 to P-421; P-266 to P-421; P-267 to P-421; H-268 to P-421; A-269 to P-421; R-270 to P-421; I-271 to P-421; S-272 to P-421; Q-273 to P-421; H-274 to P-421; G-275 to P-421; D-276 to P-421; P-277 to P-421; L-278 to P-421; L-279 to P-421; S-280 to P-421; N-281 to P-421; T-282 to P-421; F-283 to P-421; T-284 to P-421; E-285 to P-421; T-286 to P-421; N-287 to P-421; N-288 to P-421; H-289 to P-421; W-290 to P-421; P-291 to P-421; Q-292 to P-421; G-293 to P-421; P-294 to P-421; T-295 to P-421; G-296 to P-421; P-297 to P-421; P-298 to P-421; G-299 to P-421; P-300 to P-421; P-301 to P-421; G-302 to P-421; P-303 to P-421; M-304 to P-421; G-305 to P-421; P-306 to P-421; P-307 to P-421; G-308 to P-421; P-309 to P-421; P-310 to P-421; G-311 to P-421; P-312 to P-421; T-313 to P-421; G-314 to P-421; V-315 to P-421; P-316 to P-421; G-317 to P-421; S-318 to P-421; P-319 to P-421; G-320 to P-421; H-321 to P-421; I-322 to P-421; G-323 to P-421; P-324 to P-421; P-325 to P-421; G-326 to P-421; P-327 to P-421; T-328 to P-421; G-329 to P-421; P-330 to P-421; K-331 to P-421; G-332 to P-421; I-333 to P-421; S-334 to P-421; G-335 to P-421; H-336 to P-421; P-337 to P-421; G-338 to P-421; E-339 to P-421; K-340 to P-421; G-341 to P-421; E-342 to P-421; R-343 to P-421; G-344 to P-421; L-345 to P-421; R-346 to P-421; G-347 to P-421; E-348 to P-421; P-349 to P-421; G-350 to P-421; P-351 to P-421; Q-

352 to P-421; G-353 to P-421; S-354 to P-421; A-355 to P-421; G-356 to P-421; Q-357 to P-421; R-358 to P-421; G-359 to P-421; E-360 to P-421; P-361 to P-421; G-362 to P-421; P-363 to P-421; K-364 to P-421; G-365 to P-421; D-366 to P-421; P-367 to P-421; G-368 to P-421; E-369 to P-421; K-370 to P-421; S-371 to P-421; H-372 to P-421; W-373 to P-421; N-374 to P-421; Q-375 to P-421; S-376 to P-421; W-377 to P-421; G-378 to P-421; L-379 to P-421; G-380 to P-421; R-381 to P-421; A-382 to P-421; L-383 to P-421; P-384 to P-421; A-385 to P-421; Q-386 to P-421; A-387 to P-421; P-388 to P-421; P-389 to P-421; A-390 to P-421; S-391 to P-421; F-392 to P-421; G-393 to P-421; A-394 to P-421; R-395 to P-421; G-396 to P-421; A-397 to P-421; D-398 to P-421; M-399 to P-421; Q-400 to P-421; P-401 to P-421; T-402 to P-421; T-403 to P-421; G-404 to P-421; S-405 to P-421; W-406 to P-421; P-407 to P-421; P-408 to P-421; G-409 to P-421; A-410 to P-421; G-411 to P-421; T-412 to P-421; R-413 to P-421; E-414 to P-421; A-415 to P-421; and E-416 to P-421 of SEQ ID NO: 51.

[0069] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0070] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 51: R-246 to G-420; R-246 to G-419; R-246 to G-418; R-246 to G-417; R-246 to E-416; R-246 to A-415; R-246 to E-414; R-246 to R-413; R-246 to T-412; R-246 to G-411; R-246 to A-410; R-246 to G-409; R-246 to P-408; R-246 to P-407; R-246 to W-406; R-246 to S-405; R-246 to G-404; R-246 to T-403; R-246 to T-402; R-246 to P-401; R-246 to Q-400; R-246 to M-399; R-246 to D-398; R-246 to A-397; R-246 to G-396; R-246 to R-395; R-246 to A-394; R-246 to G-393; R-246 to F-392; R-246 to S-391; R-246

to A-390; R-246 to P-389; R-246 to P-388; R-246 to A-387; R-246 to Q-386; R-246 to A-385; R-246 to P-384; R-246 to L-383; R-246 to A-382; R-246 to R-381; R-246 to G-380; R-246 to L-379; R-246 to G-378; R-246 to W-377; R-246 to S-376; R-246 to Q-375; R-246 to N-374; R-246 to W-373; R-246 to H-372; R-246 to S-371; R-246 to K-370; R-246 to E-369; R-246 to G-368; R-246 to P-367; R-246 to D-366; R-246 to G-365; R-246 to K-364; R-246 to P-363; R-246 to G-362; R-246 to P-361; R-246 to E-360; R-246 to G-359; R-246 to R-358; R-246 to Q-357; R-246 to G-356; R-246 to A-355; R-246 to S-354; R-246 to G-353; R-246 to Q-352; R-246 to P-351; R-246 to G-350; R-246 to P-349; R-246 to E-348; R-246 to G-347; R-246 to R-346; R-246 to L-345; R-246 to G-344; R-246 to R-343; R-246 to E-342; R-246 to G-341; R-246 to K-340; R-246 to E-339; R-246 to G-338; R-246 to P-337; R-246 to H-336; R-246 to G-335; R-246 to S-334; R-246 to I-333; R-246 to G-332; R-246 to K-331; R-246 to P-330; R-246 to G-329; R-246 to T-328; R-246 to P-327; R-246 to G-326; R-246 to P-325; R-246 to P-324; R-246 to G-323; R-246 to I-322; R-246 to H-321; R-246 to G-320; R-246 to P-319; R-246 to S-318; R-246 to G-317; R-246 to P-316; R-246 to V-315; R-246 to G-314; R-246 to T-313; R-246 to P-312; R-246 to G-311; R-246 to P-310; R-246 to P-309; R-246 to G-308; R-246 to P-307; R-246 to P-306; R-246 to G-305; R-246 to M-304; R-246 to P-303; R-246 to G-302; R-246 to P-301; R-246 to P-300; R-246 to G-299; R-246 to P-298; R-246 to P-297; R-246 to G-296; R-246 to T-295; R-246 to P-294; R-246 to G-293; R-246 to Q-292; R-246 to P-291; R-246 to W-290; R-246 to H-289; R-246 to N-288; R-246 to N-287; R-246 to T-286; R-246 to E-285; R-246 to T-284; R-246 to F-283; R-246 to T-282; R-246 to N-281; R-246 to S-280; R-246 to L-279; R-246 to L-278; R-246 to P-277; R-246 to D-276; R-246 to G-275; R-246 to H-274; R-246 to Q-273; R-246 to S-272; R-246 to I-271; R-246 to R-270; R-246 to A-269; R-246 to H-268; R-246 to P-267; R-246 to P-266; R-246 to G-265; R-246 to V-264; R-246 to P-263; R-246 to A-262; R-246 to P-261; R-246 to P-260; R-246 to G-259; R-246 to P-258; R-246 to P-257; R-246 to G-256; R-246 to P-255; R-246 to P-254; R-246 to G-253; and R-246 to P-252 of SEQ ID NO: 51.

[0071] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under

stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0072] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 51, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0073] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-623, where this portion excludes any integer of amino acid residues from 1 to about 415 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-623, or any integer of amino acid residues from 1 to about 415 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-623. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0074] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0075] It has been discovered that this gene is expressed in the small and large intestine, stomach, lung, and prostate.

[0076] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism; obesity; and inflammation, including inflammatory disorders of the gastrointestinal tract and lungs.

[0077] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, gastrointestinal, and respiratory systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, gastrointestinal, pulmonary, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0078] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or

ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0079] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0080] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES FOR PROTEIN ENCODED BY GENE NO.: 3

[0081] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329840), and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543, AAB06706, and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0082] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703

(1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0083] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 52 as residues: Ser-11 to His-22, Ser-34 to Phe-40, Ala-66 to Phe-71, Leu-96 to Lys-104. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0084] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 52 as residues Leu-79 to Thr-240. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0085] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 52 demonstrating

functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0086] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0087] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0088] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 52: P-80 to T-240; Q-81 to T-240; G-82 to T-240; A-83 to T-240; G-84 to T-240; L-85 to T-240; R-86 to T-240; L-87 to T-240; V-88 to T-240; G-

89 to T-240; E-90 to T-240; A-91 to T-240; F-92 to T-240; H-93 to T-240; C-94 to T-240; R-95 to T-240; L-96 to T-240; Q-97 to T-240; G-98 to T-240; P-99 to T-240; R-100 to T-240; R-101 to T-240; V-102 to T-240; D-103 to T-240; K-104 to T-240; R-105 to T-240; T-106 to T-240; L-107 to T-240; V-108 to T-240; E-109 to T-240; L-110 to T-240; H-111 to T-240; G-112 to T-240; F-113 to T-240; Q-114 to T-240; A-115 to T-240; P-116 to T-240; A-117 to T-240; A-118 to T-240; Q-119 to T-240; G-120 to T-240; A-121 to T-240; F-122 to T-240; L-123 to T-240; R-124 to T-240; G-125 to T-240; S-126 to T-240; G-127 to T-240; L-128 to T-240; S-129 to T-240; L-130 to T-240; A-131 to T-240; S-132 to T-240; G-133 to T-240; R-134 to T-240; F-135 to T-240; T-136 to T-240; A-137 to T-240; P-138 to T-240; V-139 to T-240; S-140 to T-240; G-141 to T-240; I-142 to T-240; F-143 to T-240; Q-144 to T-240; F-145 to T-240; S-146 to T-240; A-147 to T-240; S-148 to T-240; L-149 to T-240; H-150 to T-240; V-151 to T-240; D-152 to T-240; H-153 to T-240; S-154 to T-240; E-155 to T-240; L-156 to T-240; Q-157 to T-240; G-158 to T-240; K-159 to T-240; A-160 to T-240; R-161 to T-240; L-162 to T-240; R-163 to T-240; A-164 to T-240; R-165 to T-240; D-166 to T-240; V-167 to T-240; V-168 to T-240; C-169 to T-240; V-170 to T-240; L-171 to T-240; I-172 to T-240; C-173 to T-240; I-174 to T-240; E-175 to T-240; S-176 to T-240; L-177 to T-240; C-178 to T-240; Q-179 to T-240; R-180 to T-240; H-181 to T-240; T-182 to T-240; C-183 to T-240; L-184 to T-240; E-185 to T-240; A-186 to T-240; V-187 to T-240; S-188 to T-240; G-189 to T-240; L-190 to T-240; E-191 to T-240; S-192 to T-240; N-193 to T-240; S-194 to T-240; R-195 to T-240; V-196 to T-240; F-197 to T-240; T-198 to T-240; L-199 to T-240; Q-200 to T-240; V-201 to T-240; Q-202 to T-240; G-203 to T-240; L-204 to T-240; L-205 to T-240; Q-206 to T-240; L-207 to T-240; Q-208 to T-240; A-209 to T-240; G-210 to T-240; Q-211 to T-240; Y-212 to T-240; A-213 to T-240; S-214 to T-240; V-215 to T-240; F-216 to T-240; V-217 to T-240; D-218 to T-240; N-219 to T-240; G-220 to T-240; S-221 to T-240; G-222 to T-240; A-223 to T-240; V-224 to T-240; L-225 to T-240; T-226 to T-240; I-227 to T-240; Q-228 to T-240; A-229 to T-240; G-230 to T-240; S-231 to T-240; S-232 to T-240; F-233 to T-240; S-234 to T-240; and G-235 to T-240 of SEQ ID NO: 52.

[0089] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these

polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0090] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 52: L-79 to G-239; L-79 to L-238; L-79 to L-237; L-79 to L-236; L-79 to G-235; L-79 to S-234; L-79 to F-233; L-79 to S-232; L-79 to S-231; L-79 to G-230; L-79 to A-229; L-79 to Q-228; L-79 to I-227; L-79 to T-226; L-79 to L-225; L-79 to V-224; L-79 to A-223; L-79 to G-222; L-79 to S-221; L-79 to G-220; L-79 to N-219; L-79 to D-218; L-79 to V-217; L-79 to F-216; L-79 to V-215; L-79 to S-214; L-79 to A-213; L-79 to Y-212; L-79 to Q-211; L-79 to G-210; L-79 to A-209; L-79 to Q-208; L-79 to L-207; L-79 to Q-206; L-79 to L-205; L-79 to L-204; L-79 to G-203; L-79 to Q-202; L-79 to V-201; L-79 to Q-200; L-79 to L-199; L-79 to T-198; L-79 to F-197; L-79 to V-196; L-79 to R-195; L-79 to S-194; L-79 to N-193; L-79 to S-192; L-79 to E-191; L-79 to L-190; L-79 to G-189; L-79 to S-188; L-79 to V-187; L-79 to A-186; L-79 to E-185; L-79 to L-184; L-79 to C-183; L-79 to T-182; L-79 to H-181; L-79 to R-180; L-79 to Q-179; L-79 to C-178; L-79 to L-177; L-79 to S-176; L-79 to E-175; L-79 to I-174; L-79 to C-173; L-79 to I-172; L-79 to L-171; L-79 to V-170; L-79 to C-169; L-79 to V-168; L-79 to V-167; L-79 to D-166; L-79 to R-165; L-79 to A-164; L-79 to R-163; L-79 to L-162; L-79 to R-161; L-79 to A-160; L-79 to K-159; L-79 to G-158; L-79 to Q-157; L-79 to L-156; L-79 to E-155; L-79 to S-154; L-79 to H-153; L-79 to D-152; L-79 to V-151; L-79 to H-150; L-79 to L-149; L-79 to S-148; L-79 to A-147; L-79 to S-146; L-79 to F-145; L-79 to Q-144; L-79 to F-143; L-79 to I-142; L-79 to G-141; L-79 to S-140; L-79 to V-139; L-79 to P-138; L-79 to A-137; L-79 to T-136; L-79 to F-135; L-79 to R-134; L-79 to G-133; L-79 to S-132; L-79 to A-131; L-79 to L-130; L-79 to S-129; L-79 to L-128; L-79 to G-127; L-79 to S-126; L-79 to G-125; L-79 to R-124; L-79 to L-123; L-79 to F-122; L-79 to A-121; L-79 to G-120; L-79 to Q-119; L-79 to A-118; L-79 to A-117; L-79 to P-116; L-79 to A-115; L-79 to Q-114; L-79 to F-113; L-79 to G-112; L-79 to H-111; L-79 to L-110; L-79 to E-109; L-79 to

V-108; L-79 to L-107; L-79 to T-106; L-79 to R-105; L-79 to K-104; L-79 to D-103; L-79 to V-102; L-79 to R-101; L-79 to R-100; L-79 to P-99; L-79 to G-98; L-79 to Q-97; L-79 to L-96; L-79 to R-95; L-79 to C-94; L-79 to H-93; L-79 to F-92; L-79 to A-91; L-79 to E-90; L-79 to G-89; L-79 to V-88; L-79 to L-87; L-79 to R-86; and L-79 to L-85 of SEQ ID NO: 52.

[0091] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0092] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 52, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0093] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0094] It has been discovered that this gene is expressed in kidney, as well as cancer tissues.

[0095] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism, obesity, as well as kidney disorders.

[0096] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and renal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, renal, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0097] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but

not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0100] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0101] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES FOR PROTEIN ENCODED BY GENE NO.: 4

[0102] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329840), and ACRP30, an adipocyte complement-related

protein (see, e.g., Genbank Accession Numbers AAA80543, AAB06706, and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0103] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding

agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0104] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 53 as residues: Gln-31 to Gly-67, Cys-77 to Ser-82, Gly-99 to Gly-117, Ala-121 to Gly-132, Pro-137 to Ser-143, Gly-151 to Tyr-162. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0105] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 53 as residues Met-134 to Pro-281. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0106] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 53 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention.

Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0107] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to a polypeptide of the invention), and ability to form multimers with polypeptides of the invention.

[0108] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0109] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 53: G-135 to P-281; A-136 to P-281; P-137 to P-281; G-138 to P-281; E-139 to P-281; R-140 to P-281; C-141 to P-281; K-142 to P-281; S-143 to P-281; H-144 to P-281; Y-145 to P-281; A-146 to P-281; A-147 to P-281; F-148 to P-281; S-149 to P-281; V-150 to P-281; G-151 to P-281; R-152 to P-281; K-153 to P-281; K-154

to P-281; P-155 to P-281; M-156 to P-281; H-157 to P-281; S-158 to P-281; N-159 to P-281; H-160 to P-281; Y-161 to P-281; Y-162 to P-281; Q-163 to P-281; T-164 to P-281; V-165 to P-281; I-166 to P-281; F-167 to P-281; D-168 to P-281; T-169 to P-281; E-170 to P-281; F-171 to P-281; V-172 to P-281; N-173 to P-281; L-174 to P-281; Y-175 to P-281; D-176 to P-281; H-177 to P-281; F-178 to P-281; N-179 to P-281; M-180 to P-281; F-181 to P-281; T-182 to P-281; G-183 to P-281; K-184 to P-281; F-185 to P-281; Y-186 to P-281; C-187 to P-281; Y-188 to P-281; V-189 to P-281; P-190 to P-281; G-191 to P-281; L-192 to P-281; Y-193 to P-281; F-194 to P-281; F-195 to P-281; S-196 to P-281; L-197 to P-281; N-198 to P-281; V-199 to P-281; H-200 to P-281; T-201 to P-281; W-202 to P-281; N-203 to P-281; Q-204 to P-281; K-205 to P-281; E-206 to P-281; T-207 to P-281; Y-208 to P-281; L-209 to P-281; H-210 to P-281; I-211 to P-281; M-212 to P-281; K-213 to P-281; N-214 to P-281; E-215 to P-281; E-216 to P-281; E-217 to P-281; V-218 to P-281; A-219 to P-281; I-220 to P-281; L-221 to P-281; F-222 to P-281; A-223 to P-281; Q-224 to P-281; V-225 to P-281; G-226 to P-281; D-227 to P-281; R-228 to P-281; S-229 to P-281; I-230 to P-281; M-231 to P-281; Q-232 to P-281; S-233 to P-281; Q-234 to P-281; S-235 to P-281; L-236 to P-281; M-237 to P-281; L-238 to P-281; E-239 to P-281; L-240 to P-281; R-241 to P-281; E-242 to P-281; Q-243 to P-281; D-244 to P-281; Q-245 to P-281; V-246 to P-281; W-247 to P-281; V-248 to P-281; R-249 to P-281; L-250 to P-281; Y-251 to P-281; K-252 to P-281; G-253 to P-281; E-254 to P-281; R-255 to P-281; E-256 to P-281; N-257 to P-281; A-258 to P-281; I-259 to P-281; F-260 to P-281; S-261 to P-281; E-262 to P-281; E-263 to P-281; L-264 to P-281; D-265 to P-281; T-266 to P-281; Y-267 to P-281; I-268 to P-281; T-269 to P-281; F-270 to P-281; S-271 to P-281; G-272 to P-281; Y-273 to P-281; L-274 to P-281; V-275 to P-281; K-276 to P-281; of SEQ ID NO: 53.

[0110] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention.

Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0111] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 53: M-134 to E-280; M-134 to T-279; M-134 to A-278; M-134 to H-277; M-134 to K-276; M-134 to V-275; M-134 to L-274; M-134 to Y-273; M-134 to G-272; M-134 to S-271; M-134 to F-270; M-134 to T-269; M-134 to I-268; M-134 to Y-267; M-134 to T-266; M-134 to D-265; M-134 to L-264; M-134 to E-263; M-134 to E-262; M-134 to S-261; M-134 to F-260; M-134 to I-259; M-134 to A-258; M-134 to N-257; M-134 to E-256; M-134 to R-255; M-134 to E-254; M-134 to G-253; M-134 to K-252; M-134 to Y-251; M-134 to L-250; M-134 to R-249; M-134 to V-248; M-134 to W-247; M-134 to V-246; M-134 to Q-245; M-134 to D-244; M-134 to Q-243; M-134 to E-242; M-134 to R-241; M-134 to L-240; M-134 to E-239; M-134 to L-238; M-134 to M-237; M-134 to L-236; M-134 to S-235; M-134 to Q-234; M-134 to S-233; M-134 to Q-232; M-134 to M-231; M-134 to I-230; M-134 to S-229; M-134 to R-228; M-134 to D-227; M-134 to G-226; M-134 to V-225; M-134 to Q-224; M-134 to A-223; M-134 to F-222; M-134 to L-221; M-134 to I-220; M-134 to A-219; M-134 to V-218; M-134 to E-217; M-134 to E-216; M-134 to E-215; M-134 to N-214; M-134 to K-213; M-134 to M-212; M-134 to I-211; M-134 to H-210; M-134 to L-209; M-134 to Y-208; M-134 to T-207; M-134 to E-206; M-134 to K-205; M-134 to Q-204; M-134 to N-203; M-134 to W-202; M-134 to T-201; M-134 to H-200; M-134 to V-199; M-134 to N-198; M-134 to L-197; M-134 to S-196; M-134 to F-195; M-134 to F-194; M-134 to Y-193; M-134 to L-192; M-134 to G-191; M-134 to P-190; M-134 to V-189; M-134 to Y-188; M-134 to C-187; M-134 to Y-186; M-134 to F-185; M-134 to K-184; M-134 to G-183; M-134 to T-182; M-134 to F-181; M-134 to M-180; M-134 to N-179; M-134 to F-178; M-134 to H-177; M-134 to D-176; M-134 to Y-175; M-134 to L-174; M-134 to N-173; M-134 to V-172; M-134 to F-171; M-134 to E-170; M-134 to T-169; M-134 to D-168; M-134 to F-167; M-134 to I-166; M-134 to V-165; M-134 to T-164; M-134 to Q-163; M-134 to Y-162; M-134 to Y-161; M-134 to H-160; M-134 to N-159; M-134 to S-158; M-134 to H-157; M-134 to M-156; M-134 to P-155; M-134 to K-154; M-134 to K-153; M-134 to R-152; M-134 to G-151; M-134 to V-150; M-134 to S-149; M-134 to F-148; M-134 to A-147; M-134 to A-146; M-

134 to Y-145; M-134 to H-144; M-134 to S-143; M-134 to K-142; M-134 to C-141; and M-134 to R-140 of SEQ ID NO: 53.

[0112] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0113] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 53, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0114] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-536, where this portion excludes any integer of amino acid residues from 1 to about 275 amino acids from the amino terminus of the complete amino

acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-536, or any integer of amino acid residues from 1 to about 275 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-536. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0115] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0116] It has been discovered that this gene is expressed in adult and fetal heart, pancreas, liver, and human cerebellum.

[0117] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism, obesity, as well as cardiovascular, immunological and neurological disorders.

[0118] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, cardiovascular, immune, and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, vascular, immune, neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0119] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including

antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0120] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0121] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 5

[0122] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including precerebellin (see, e.g., Swiss-Prot Accession Q9JHG0), which is involved in neural functions such as the control of food intake and catecholamine release, and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0123] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular

fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0124] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 54 as residues Gly-60 to Leu-205. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0125] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 54 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0126] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological

activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0127] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0128] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 54: G-61 to L-205; A-62 to L-205; A-63 to L-205; L-64 to L-205; G-65 to L-205; E-66 to L-205; A-67 to L-205; P-68 to L-205; P-69 to L-205; G-70 to L-205; R-71 to L-205; V-72 to L-205; A-73 to L-205; F-74 to L-205; A-75 to L-205; A-76 to L-205; V-77 to L-205; R-78 to L-205; S-79 to L-205; H-80 to L-205; H-81 to L-205; H-82 to L-205; E-83 to L-205; P-84 to L-205; A-85 to L-205; G-86 to L-205; E-87 to L-205; T-88 to L-205; G-89 to L-205; N-90 to L-205; G-91 to L-205; T-92 to L-205; S-93 to L-205; G-94 to L-205; A-95 to L-205; I-96 to L-205; Y-97 to L-205; F-98 to L-205; D-99 to L-205; Q-100 to L-205; V-101 to L-205; L-102 to L-205; V-103 to L-205; N-104 to L-205; E-105 to L-205; G-106 to L-205; G-107 to L-205; G-108 to L-205; F-109 to L-

205; D-110 to L-205; R-111 to L-205; A-112 to L-205; S-113 to L-205; G-114 to L-205; S-115 to L-205; F-116 to L-205; V-117 to L-205; A-118 to L-205; P-119 to L-205; V-120 to L-205; R-121 to L-205; G-122 to L-205; V-123 to L-205; Y-124 to L-205; S-125 to L-205; F-126 to L-205; R-127 to L-205; F-128 to L-205; H-129 to L-205; V-130 to L-205; V-131 to L-205; K-132 to L-205; V-133 to L-205; Y-134 to L-205; N-135 to L-205; R-136 to L-205; Q-137 to L-205; T-138 to L-205; V-139 to L-205; Q-140 to L-205; V-141 to L-205; S-142 to L-205; L-143 to L-205; M-144 to L-205; L-145 to L-205; N-146 to L-205; T-147 to L-205; W-148 to L-205; P-149 to L-205; V-150 to L-205; I-151 to L-205; S-152 to L-205; A-153 to L-205; F-154 to L-205; A-155 to L-205; N-156 to L-205; D-157 to L-205; P-158 to L-205; D-159 to L-205; V-160 to L-205; T-161 to L-205; R-162 to L-205; E-163 to L-205; A-164 to L-205; A-165 to L-205; T-166 to L-205; S-167 to L-205; S-168 to L-205; V-169 to L-205; L-170 to L-205; L-171 to L-205; P-172 to L-205; L-173 to L-205; D-174 to L-205; P-175 to L-205; G-176 to L-205; D-177 to L-205; R-178 to L-205; V-179 to L-205; S-180 to L-205; L-181 to L-205; R-182 to L-205; L-183 to L-205; R-184 to L-205; R-185 to L-205; G-186 to L-205; N-187 to L-205; L-188 to L-205; L-189 to L-205; G-190 to L-205; G-191 to L-205; W-192 to L-205; K-193 to L-205; Y-194 to L-205; S-195 to L-205; S-196 to L-205; F-197 to L-205; S-198 to L-205; G-199 to L-205; and F-200 to L-205 of SEQ ID NO: 54.

[0129] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0130] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 54: G-60 to P-204; G-60 to F-203; G-60 to I-202; G-60 to L-201; G-60 to

F-200; G-60 to G-199; G-60 to S-198; G-60 to F-197; G-60 to S-196; G-60 to S-195; G-60 to Y-194; G-60 to K-193; G-60 to W-192; G-60 to G-191; G-60 to G-190; G-60 to L-189; G-60 to L-188; G-60 to N-187; G-60 to G-186; G-60 to R-185; G-60 to R-184; G-60 to L-183; G-60 to R-182; G-60 to L-181; G-60 to S-180; G-60 to V-179; G-60 to R-178; G-60 to D-177; G-60 to G-176; G-60 to P-175; G-60 to D-174; G-60 to L-173; G-60 to P-172; G-60 to L-171; G-60 to L-170; G-60 to V-169; G-60 to S-168; G-60 to S-167; G-60 to T-166; G-60 to A-165; G-60 to A-164; G-60 to E-163; G-60 to R-162; G-60 to T-161; G-60 to V-160; G-60 to D-159; G-60 to P-158; G-60 to D-157; G-60 to N-156; G-60 to A-155; G-60 to F-154; G-60 to A-153; G-60 to S-152; G-60 to I-151; G-60 to V-150; G-60 to P-149; G-60 to W-148; G-60 to T-147; G-60 to N-146; G-60 to L-145; G-60 to M-144; G-60 to L-143; G-60 to S-142; G-60 to V-141; G-60 to Q-140; G-60 to V-139; G-60 to T-138; G-60 to Q-137; G-60 to R-136; G-60 to N-135; G-60 to Y-134; G-60 to V-133; G-60 to K-132; G-60 to V-131; G-60 to V-130; G-60 to H-129; G-60 to F-128; G-60 to R-127; G-60 to F-126; G-60 to S-125; G-60 to Y-124; G-60 to V-123; G-60 to G-122; G-60 to R-121; G-60 to V-120; G-60 to P-119; G-60 to A-118; G-60 to V-117; G-60 to F-116; G-60 to S-115; G-60 to G-114; G-60 to S-113; G-60 to A-112; G-60 to R-111; G-60 to D-110; G-60 to F-109; G-60 to G-108; G-60 to G-107; G-60 to G-106; G-60 to E-105; G-60 to N-104; G-60 to V-103; G-60 to L-102; G-60 to V-101; G-60 to Q-100; G-60 to D-99; G-60 to F-98; G-60 to Y-97; G-60 to I-96; G-60 to A-95; G-60 to G-94; G-60 to S-93; G-60 to T-92; G-60 to G-91; G-60 to N-90; G-60 to G-89; G-60 to T-88; G-60 to E-87; G-60 to G-86; G-60 to A-85; G-60 to P-84; G-60 to E-83; G-60 to H-82; G-60 to H-81; G-60 to H-80; G-60 to S-79; G-60 to R-78; G-60 to V-77; G-60 to A-76; G-60 to A-75; G-60 to F-74; G-60 to A-73; G-60 to V-72; G-60 to R-71; G-60 to G-70; G-60 to P-69; G-60 to P-68; G-60 to A-67; and G-60 to E-66 of SEQ ID NO: 54.

[0131] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention.

Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0132] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 54, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0133] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0134] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, where this portion excludes any integer of amino acid residues from 1 to about 199 amino acids from the amino terminus of the complete amino

acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, or any integer of amino acid residues from 1 to about 199 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-3696. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0135] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0136] It has been discovered that this gene is expressed in heart tissue, as well as human cerebellum, infant brain, fetal lung and spleen.

[0137] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism, as well as cardiovascular, immunological and neurological disorders.

[0138] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, cardiovascular, immune, and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, cardiovascular, immune, neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0139] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including

antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0140] The expression of this gene in heart tissue, and the similarity of this gene to other members of the C1q family of proteins, suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of cardiovascular disorders (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below).

[0141] In addition, expression of this gene in lung and spleen tissue, and the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune

diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0142] More generally, the expression of this gene in human cerebellum and infant brain and homology to precerebellin (Swiss-Prot Accession Q9JHG0) indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of neurological disorders, such as those described herein under "Neural Activity and Neurological Diseases".

[0143] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 6

[0144] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a cerebellin-like glycoprotein (see, e.g. Genbank Accession No. A60032), which is involved in neural functions such as the control of food intake and catecholamine release; and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between

C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0145] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0146] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 55 as residues Leu-47 to Leu-189. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention.

Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0147] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 55 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0148] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0149] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0150] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 55: G-48 to L-189; I-49 to L-189; S-50 to L-189; V-51 to L-189; R-52 to L-189; S-53 to L-189; G-54 to L-189; S-55 to L-189; A-56 to L-189; K-57 to L-189; V-58 to L-189; A-59 to L-189; F-60 to L-189; S-61 to L-189; A-62 to L-189; T-63 to L-189; R-64 to L-189; S-65 to L-189; T-66 to L-189; N-67 to L-189; H-68 to L-189; E-69 to L-189; P-70 to L-189; S-71 to L-189; E-72 to L-189; M-73 to L-189; S-74 to L-189; N-75 to L-189; R-76 to L-189; T-77 to L-189; M-78 to L-189; T-79 to L-189; I-80 to L-189; Y-81 to L-189; F-82 to L-189; D-83 to L-189; Q-84 to L-189; V-85 to L-189; L-86 to L-189; V-87 to L-189; N-88 to L-189; I-89 to L-189; G-90 to L-189; N-91 to L-189; H-92 to L-189; F-93 to L-189; D-94 to L-189; L-95 to L-189; A-96 to L-189; S-97 to L-189; S-98 to L-189; I-99 to L-189; F-100 to L-189; V-101 to L-189; A-102 to L-189; P-103 to L-189; R-104 to L-189; K-105 to L-189; G-106 to L-189; I-107 to L-189; Y-108 to L-189; S-109 to L-189; F-110 to L-189; S-111 to L-189; F-112 to L-189; H-113 to L-189; V-114 to L-189; V-115 to L-189; K-116 to L-189; V-117 to L-189; Y-118 to L-189; N-119 to L-189; R-120 to L-189; Q-121 to L-189; T-122 to L-189; I-123 to L-189; Q-124 to L-189; V-125 to L-189; S-126 to L-189; L-127 to L-189; M-128 to L-189; Q-129 to L-189; N-130 to L-189; G-131 to L-189; Y-132 to L-189; P-133 to L-189; V-134 to L-189; I-135 to L-189; S-136 to L-189; A-137 to L-189; F-138 to L-189; A-139 to L-189; G-140 to L-189; D-141 to L-189; Q-142 to L-189; D-143 to L-189; V-144 to L-189; T-145 to L-189; R-146 to L-189; E-147 to L-189; A-148 to L-189; A-149 to L-189; S-150 to L-189; N-151 to L-189; G-152 to L-189; V-153 to L-189; L-154 to L-189; L-155 to L-189; L-156 to L-189; M-157 to L-189; E-158 to L-189; R-159 to L-189; E-160 to L-189; D-161 to L-189; K-162 to L-189; V-163 to L-189; H-164 to L-189; L-165 to L-189; K-166 to L-189; L-167 to L-189; E-168 to L-189; R-169 to L-189; G-170 to L-189; N-171 to L-189; L-172 to L-189; M-173 to L-189; G-174 to L-189; G-175 to L-189; W-176 to L-189; K-177 to L-189; Y-178 to L-189; S-179 to L-189; T-180 to L-189; F-181 to L-189; S-182 to L-189; G-183 to L-189; and F-184 to L-189 of SEQ ID NO: 55.

[0151] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein,

polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0152] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 55: L-47 to P-188; L-47 to F-187; L-47 to V-186; L-47 to L-185; L-47 to F-184; L-47 to G-183; L-47 to S-182; L-47 to F-181; L-47 to T-180; L-47 to S-179; L-47 to Y-178; L-47 to K-177; L-47 to W-176; L-47 to G-175; L-47 to G-174; L-47 to M-173; L-47 to L-172; L-47 to N-171; L-47 to G-170; L-47 to R-169; L-47 to E-168; L-47 to L-167; L-47 to K-166; L-47 to L-165; L-47 to H-164; L-47 to V-163; L-47 to K-162; L-47 to D-161; L-47 to E-160; L-47 to R-159; L-47 to E-158; L-47 to M-157; L-47 to L-156; L-47 to L-155; L-47 to L-154; L-47 to V-153; L-47 to G-152; L-47 to N-151; L-47 to S-150; L-47 to A-149; L-47 to A-148; L-47 to E-147; L-47 to R-146; L-47 to T-145; L-47 to V-144; L-47 to D-143; L-47 to Q-142; L-47 to D-141; L-47 to G-140; L-47 to A-139; L-47 to F-138; L-47 to A-137; L-47 to S-136; L-47 to I-135; L-47 to V-134; L-47 to P-133; L-47 to Y-132; L-47 to G-131; L-47 to N-130; L-47 to Q-129; L-47 to M-128; L-47 to L-127; L-47 to S-126; L-47 to V-125; L-47 to Q-124; L-47 to I-123; L-47 to T-122; L-47 to Q-121; L-47 to R-120; L-47 to N-119; L-47 to Y-118; L-47 to V-117; L-47 to K-116; L-47 to V-115; L-47 to V-114; L-47 to H-113; L-47 to F-112; L-47 to S-111; L-47 to F-110; L-47 to S-109; L-47 to Y-108; L-47 to I-107; L-47 to G-106; L-47 to K-105; L-47 to R-104; L-47 to P-103; L-47 to A-102; L-47 to V-101; L-47 to F-100; L-47 to I-99; L-47 to S-98; L-47 to S-97; L-47 to A-96; L-47 to L-95; L-47 to D-94; L-47 to F-93; L-47 to H-92; L-47 to N-91; L-47 to G-90; L-47 to I-89; L-47 to N-88; L-47 to V-87; L-47 to L-86; L-47 to V-85; L-47 to Q-84; L-47 to D-83; L-47 to F-82; L-47 to Y-81; L-47 to I-80; L-47 to T-79; L-47 to M-78; L-47 to T-77; L-47 to R-76; L-47 to N-75; L-47 to S-74; L-47 to M-73; L-47 to E-72; L-47 to S-71; L-47 to P-70; L-47 to E-69; L-47 to H-68; L-47 to N-67; L-47 to T-66; L-47 to S-65; L-47 to R-64; L-47 to T-63; L-47 to A-62; L-47 to S-61; L-47 to F-60;

L-47 to A-59; L-47 to V-58; L-47 to K-57; L-47 to A-56; L-47 to S-55; L-47 to G-54; and L-47 to S-53 of SEQ ID NO: 55.

[0153] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0154] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 55, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0155] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%,

98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0156] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, where this portion excludes any integer of amino acid residues from 1 to about 183 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, or any integer of amino acid residues from 1 to about 183 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0157] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0158] Translation products of this gene stimulate glucose transport in adipocytes.

[0159] It has been discovered that this gene is expressed in neural tissues, including infant/fetal brain, and human hypothalamus.

[0160] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism, obesity, as well as neurological disorders.

[0161] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, neural,

cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0162] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0163] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene,

including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0164] More generally, the expression of this gene in infant/fetal brain and human hypothalamus and homology to a cerebellin-like glycoprotein indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of neurological disorders, such as those described herein under "Neural Activity and Neurological Diseases".

[0165] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 7

[0166] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329841), and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and

energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0167] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0168] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, or all four of the immunogenic epitopes shown in SEQ ID NO: 56 as residues: Pro-5 to Gly-15, Pro-20 to Gly-27, Pro-32 to Glu-43, and Ala-66 to Ser-77. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are

encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0169] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 56 as residues Thr-35 to Ala-201. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0170] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 56 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0171] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0172] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g.,

ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0173] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 56: G-51 to A-201; P-52 to A-201; A-53 to A-201; G-54 to A-201; E-55 to A-201; C-56 to A-201; S-57 to A-201; V-58 to A-201; P-59 to A-201; P-60 to A-201; R-61 to A-201; S-62 to A-201; A-63 to A-201; F-64 to A-201; S-65 to A-201; A-66 to A-201; K-67 to A-201; R-68 to A-201; S-69 to A-201; E-70 to A-201; S-71 to A-201; R-72 to A-201; V-73 to A-201; P-74 to A-201; P-75 to A-201; P-76 to A-201; S-77 to A-201; D-78 to A-201; A-79 to A-201; P-80 to A-201; L-81 to A-201; P-82 to A-201; F-83 to A-201; D-84 to A-201; R-85 to A-201; V-86 to A-201; L-87 to A-201; V-88 to A-201; N-89 to A-201; E-90 to A-201; Q-91 to A-201; G-92 to A-201; H-93 to A-201; Y-94 to A-201; D-95 to A-201; A-96 to A-201; V-97 to A-201; T-98 to A-201; G-99 to A-201; K-100 to A-201; F-101 to A-201; T-102 to A-201; C-103 to A-201; Q-104 to A-201; V-105 to A-201; P-106 to A-201; G-107 to A-201; V-108 to A-201; Y-109 to A-201; Y-110 to A-201; F-111 to A-201; A-112 to A-201; V-113 to A-201; H-114 to A-201; A-115 to A-201; T-116 to A-201; V-117 to A-201; Y-118 to A-201; R-119 to A-201; A-120 to A-201; S-121 to A-201; L-122 to A-201; Q-123 to A-201; F-124 to A-201; D-125 to A-201; L-126 to A-201; V-127 to A-201; K-128 to A-201; N-129 to A-201; G-130 to A-201; E-131 to A-201; S-132 to A-201; I-133 to A-201; A-134 to A-201; S-135 to A-201; F-136 to A-201; F-137 to A-201; Q-138 to A-201; F-139 to A-201; F-140 to A-201; G-141 to A-

201; G-142 to A-201; W-143 to A-201; P-144 to A-201; K-145 to A-201; P-146 to A-201; A-147 to A-201; S-148 to A-201; L-149 to A-201; S-150 to A-201; G-151 to A-201; G-152 to A-201; A-153 to A-201; M-154 to A-201; V-155 to A-201; R-156 to A-201; L-157 to A-201; E-158 to A-201; P-159 to A-201; E-160 to A-201; D-161 to A-201; Q-162 to A-201; V-163 to A-201; W-164 to A-201; V-165 to A-201; Q-166 to A-201; V-167 to A-201; G-168 to A-201; V-169 to A-201; G-170 to A-201; D-171 to A-201; Y-172 to A-201; I-173 to A-201; G-174 to A-201; I-175 to A-201; Y-176 to A-201; A-177 to A-201; S-178 to A-201; I-179 to A-201; K-180 to A-201; T-181 to A-201; D-182 to A-201; S-183 to A-201; T-184 to A-201; F-185 to A-201; S-186 to A-201; G-187 to A-201; F-188 to A-201; L-189 to A-201; V-190 to A-201; Y-191 to A-201; S-192 to A-201; D-193 to A-201; W-194 to A-201; H-195 to A-201; and S-196 to A-201 of SEQ ID NO: 56.

[0174] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0175] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 56: T-50 to F-200; T-50 to V-199; T-50 to P-198; T-50 to S-197; T-50 to S-196; T-50 to H-195; T-50 to W-194; T-50 to D-193; T-50 to S-192; T-50 to Y-191; T-50 to V-190; T-50 to L-189; T-50 to F-188; T-50 to G-187; T-50 to S-186; T-50 to F-185; T-50 to T-184; T-50 to S-183; T-50 to D-182; T-50 to T-181; T-50 to K-180; T-50 to I-179; T-50 to S-178; T-50 to A-177; T-50 to Y-176; T-50 to I-175; T-50 to G-174; T-50 to I-173; T-50 to Y-172; T-50 to D-171; T-50 to G-170; T-50 to V-169; T-50 to G-168; T-50 to V-167; T-50 to Q-166; T-50 to V-165; T-50 to W-164; T-50 to V-163; T-50 to Q-162; T-50 to D-161; T-50 to E-160; T-50 to P-159; T-50 to E-158; T-50 to L-157; T-50 to R-

156; T-50 to V-155; T-50 to M-154; T-50 to A-153; T-50 to G-152; T-50 to G-151; T-50 to S-150; T-50 to L-149; T-50 to S-148; T-50 to A-147; T-50 to P-146; T-50 to K-145; T-50 to P-144; T-50 to W-143; T-50 to G-142; T-50 to G-141; T-50 to F-140; T-50 to F-139; T-50 to Q-138; T-50 to F-137; T-50 to F-136; T-50 to S-135; T-50 to A-134; T-50 to I-133; T-50 to S-132; T-50 to E-131; T-50 to G-130; T-50 to N-129; T-50 to K-128; T-50 to V-127; T-50 to L-126; T-50 to D-125; T-50 to F-124; T-50 to Q-123; T-50 to L-122; T-50 to S-121; T-50 to A-120; T-50 to R-119; T-50 to Y-118; T-50 to V-117; T-50 to T-116; T-50 to A-115; T-50 to H-114; T-50 to V-113; T-50 to A-112; T-50 to F-111; T-50 to Y-110; T-50 to Y-109; T-50 to V-108; T-50 to G-107; T-50 to P-106; T-50 to V-105; T-50 to Q-104; T-50 to C-103; T-50 to T-102; T-50 to F-101; T-50 to K-100; T-50 to G-99; T-50 to T-98; T-50 to V-97; T-50 to A-96; T-50 to D-95; T-50 to Y-94; T-50 to H-93; T-50 to G-92; T-50 to Q-91; T-50 to E-90; T-50 to N-89; T-50 to V-88; T-50 to L-87; T-50 to V-86; T-50 to R-85; T-50 to D-84; T-50 to F-83; T-50 to P-82; T-50 to L-81; T-50 to P-80; T-50 to A-79; T-50 to D-78; T-50 to S-77; T-50 to P-76; T-50 to P-75; T-50 to P-74; T-50 to V-73; T-50 to R-72; T-50 to S-71; T-50 to E-70; T-50 to S-69; T-50 to R-68; T-50 to K-67; T-50 to A-66; T-50 to S-65; T-50 to F-64; T-50 to A-63; T-50 to S-62; T-50 to R-61; T-50 to P-60; T-50 to P-59; T-50 to V-58; T-50 to S-57; T-50 to C-56; of SEQ ID NO: 56.

[0176] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0177] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-

terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 56, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0178] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, where this portion excludes any integer of amino acid residues from 1 to about 195 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, or any integer of amino acid residues from 1 to about 195 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-3696. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0179] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0180] It has been discovered that this gene is expressed in fetal lung, colon tumor, fetal heart, human synovium, and human epididymus.

[0181] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism, obesity, arthritis, inflammation, and immune system dysfunction.

[0182] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, metabolic, and immune systems expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0183] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In

additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0184] In addition, the similarity of this gene to other members of the C1q family of proteins, including a TNF-related protein, suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0185] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 8

[0186] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including the complement subcomponent C1q chain C precursor (PIR Accession No. S14351), and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q

families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0187] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0188] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 57 as residues: Arg-25 to Gly-31, Pro-45 to Gly-52, Pro-71 to Gly-76, Pro-81 to Gly-91, Glu-107 to Phe-118, Thr-125 to Pro-134, Pro-147 to Gly-156, Gly-194 to Asn-203 . Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and

polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0189] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 57 as residues Pro-108 to Asp-245. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0190] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 57 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0191] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0192] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0193] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 57: G-109 to D-245; E-110 to D-245; E-111 to D-245; G-112 to D-245; R-113 to D-245; Y-114 to D-245; K-115 to D-245; Q-116 to D-245; K-117 to D-245; F-118 to D-245; Q-119 to D-245; S-120 to D-245; V-121 to D-245; F-122 to D-245; T-123 to D-245; V-124 to D-245; T-125 to D-245; R-126 to D-245; Q-127 to D-245; T-128 to D-245; H-129 to D-245; Q-130 to D-245; P-131 to D-245; P-132 to D-245; A-133 to D-245; P-134 to D-245; N-135 to D-245; S-136 to D-245; L-137 to D-245; I-138 to D-245; R-139 to D-245; F-140 to D-245; N-141 to D-245; A-142 to D-245; V-143 to D-245; L-144 to D-245; T-145 to D-245; N-146 to D-245; P-147 to D-245; Q-148 to D-245; G-149 to D-245; D-150 to D-245; Y-151 to D-245; D-152 to D-245; T-153 to D-245; S-154 to D-245; T-155 to D-245; G-156 to D-245; K-157 to D-245; F-158 to D-245; T-159 to D-245; C-160 to D-245; K-161 to D-245; V-162 to D-245; P-163 to D-245; G-164 to D-245; L-165 to D-245; Y-166 to D-245; Y-167 to D-245; F-168 to D-245; V-169 to D-245; Y-170 to D-245; H-171 to D-245; A-172 to D-245; S-173 to D-245; H-174 to D-245; T-175 to D-245; A-176 to D-245; N-177 to D-245; L-178 to D-245; C-179 to D-245; V-180 to D-245; L-181 to D-245; L-182 to D-245; Y-183 to D-245; R-184 to D-245; S-185

to D-245; G-186 to D-245; V-187 to D-245; K-188 to D-245; V-189 to D-245; V-190 to D-245; T-191 to D-245; F-192 to D-245; C-193 to D-245; G-194 to D-245; H-195 to D-245; T-196 to D-245; S-197 to D-245; K-198 to D-245; T-199 to D-245; N-200 to D-245; Q-201 to D-245; V-202 to D-245; N-203 to D-245; S-204 to D-245; G-205 to D-245; G-206 to D-245; V-207 to D-245; L-208 to D-245; L-209 to D-245; R-210 to D-245; L-211 to D-245; Q-212 to D-245; V-213 to D-245; G-214 to D-245; E-215 to D-245; E-216 to D-245; V-217 to D-245; W-218 to D-245; L-219 to D-245; A-220 to D-245; V-221 to D-245; N-222 to D-245; D-223 to D-245; Y-224 to D-245; Y-225 to D-245; D-226 to D-245; M-227 to D-245; V-228 to D-245; G-229 to D-245; I-230 to D-245; Q-231 to D-245; G-232 to D-245; S-233 to D-245; D-234 to D-245; S-235 to D-245; V-236 to D-245; F-237 to D-245; S-238 to D-245; G-239 to D-245; and F-240 to D-245 of SEQ ID NO: 57.

[0194] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0195] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 57: P-108 to P-244; P-108 to F-243; P-108 to L-242; P-108 to L-241; P-108 to F-240; P-108 to G-239; P-108 to S-238; P-108 to F-237; P-108 to V-236; P-108 to S-235; P-108 to D-234; P-108 to S-233; P-108 to G-232; P-108 to Q-231; P-108 to I-230; P-108 to G-229; P-108 to V-228; P-108 to M-227; P-108 to D-226; P-108 to Y-225; P-108 to Y-224; P-108 to D-223; P-108 to N-222; P-108 to V-221; P-108 to A-220; P-108 to L-219; P-108 to W-218; P-108 to V-217; P-108 to E-216; P-108 to E-215; P-108 to G-214; P-108 to V-213; P-108 to Q-212; P-108 to L-211; P-108 to R-210; P-108 to L-209; P-108 to L-208; P-108 to V-207; P-108 to G-206; P-108 to G-205; P-108 to S-204; P-108 to N-

203; P-108 to V-202; P-108 to Q-201; P-108 to N-200; P-108 to T-199; P-108 to K-198; P-108 to S-197; P-108 to T-196; P-108 to H-195; P-108 to G-194; P-108 to C-193; P-108 to F-192; P-108 to T-191; P-108 to V-190; P-108 to V-189; P-108 to K-188; P-108 to V-187; P-108 to G-186; P-108 to S-185; P-108 to R-184; P-108 to Y-183; P-108 to L-182; P-108 to L-181; P-108 to V-180; P-108 to C-179; P-108 to L-178; P-108 to N-177; P-108 to A-176; P-108 to T-175; P-108 to H-174; P-108 to S-173; P-108 to A-172; P-108 to H-171; P-108 to Y-170; P-108 to V-169; P-108 to F-168; P-108 to Y-167; P-108 to Y-166; P-108 to L-165; P-108 to G-164; P-108 to P-163; P-108 to V-162; P-108 to K-161; P-108 to C-160; P-108 to T-159; P-108 to F-158; P-108 to K-157; P-108 to G-156; P-108 to T-155; P-108 to S-154; P-108 to T-153; P-108 to D-152; P-108 to Y-151; P-108 to D-150; P-108 to G-149; P-108 to Q-148; P-108 to P-147; P-108 to N-146; P-108 to T-145; P-108 to L-144; P-108 to V-143; P-108 to A-142; P-108 to N-141; P-108 to F-140; P-108 to R-139; P-108 to I-138; P-108 to L-137; P-108 to S-136; P-108 to N-135; P-108 to P-134; P-108 to A-133; P-108 to P-132; P-108 to P-131; P-108 to Q-130; P-108 to H-129; P-108 to T-128; P-108 to Q-127; P-108 to R-126; P-108 to T-125; P-108 to V-124; P-108 to T-123; P-108 to F-122; P-108 to V-121; P-108 to S-120; P-108 to Q-119; P-108 to F-118; P-108 to K-117; P-108 to Q-116; P-108 to K-115; and P-108 to Y-114 of SEQ ID NO: 57.

[0196] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0197] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the

general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 57, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0198] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, where this portion excludes any integer of amino acid residues from 1 to about 239 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, or any integer of amino acid residues from 1 to about 239 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-3696. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0199] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0200] It has been discovered that this gene is expressed in spleen, as well as dendritic cells, and adult pulmonary tissues.

[0201] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism, metabolic disorders, obesity, and immune system disorders.

[0202] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, metabolic, and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0203] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In

additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0204] In addition, the expression of this gene in dendritic cells, spleen, and adult pulmonary tissues, and the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0205] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 9

[0206] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329842), and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested

that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0207] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0208] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 58 as residues: Asp-55 to Asp-67, Ser-76 to His-81, Lys-96 to Gly-103, Met-111 to Gly-133, Gln-222 to Ile-228, and Lys-250 to Tyr-258. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the

polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0209] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 58 as residues Met-132 to Asp-278. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0210] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 58 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0211] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0212] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0213] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 58: G-133 to D-278; S-134 to D-278; P-135 to D-278; G-136 to D-278; A-137 to D-278; P-138 to D-278; C-139 to D-278; Q-140 to D-278; K-141 to D-278; R-142 to D-278; F-143 to D-278; F-144 to D-278; A-145 to D-278; F-146 to D-278; S-147 to D-278; V-148 to D-278; G-149 to D-278; R-150 to D-278; K-151 to D-278; T-152 to D-278; A-153 to D-278; L-154 to D-278; H-155 to D-278; S-156 to D-278; G-157 to D-278; E-158 to D-278; D-159 to D-278; F-160 to D-278; Q-161 to D-278; T-162 to D-278; L-163 to D-278; L-164 to D-278; F-165 to D-278; E-166 to D-278; R-167 to D-278; V-168 to D-278; F-169 to D-278; V-170 to D-278; N-171 to D-278; L-172 to D-278; D-173 to D-278; G-174 to D-278; C-175 to D-278; F-176 to D-278; D-177 to D-278; M-178 to D-278; A-179 to D-278; T-180 to D-278; G-181 to D-278; Q-182 to D-278; F-183 to D-278; A-184 to D-278; A-185 to D-278; P-186 to D-278; L-187 to D-278; R-188 to D-278; G-189 to D-278; I-190 to D-278; Y-191 to D-278; F-192 to D-278; F-193 to D-278; S-194 to D-278; L-195 to D-278; N-196 to D-278; V-197 to D-278; H-198 to D-278; S-199 to D-278; W-200 to D-278; N-201 to D-278; Y-202 to D-278; K-203 to D-278; E-204 to D-278; T-205 to D-278; Y-206 to D-278; V-207 to D-278; H-208 to D-278; I-209 to D-

278; M-210 to D-278; H-211 to D-278; N-212 to D-278; Q-213 to D-278; K-214 to D-278; E-215 to D-278; A-216 to D-278; V-217 to D-278; I-218 to D-278; L-219 to D-278; Y-220 to D-278; A-221 to D-278; Q-222 to D-278; P-223 to D-278; S-224 to D-278; E-225 to D-278; R-226 to D-278; S-227 to D-278; I-228 to D-278; M-229 to D-278; Q-230 to D-278; S-231 to D-278; Q-232 to D-278; S-233 to D-278; V-234 to D-278; M-235 to D-278; L-236 to D-278; D-237 to D-278; L-238 to D-278; A-239 to D-278; Y-240 to D-278; G-241 to D-278; D-242 to D-278; R-243 to D-278; V-244 to D-278; W-245 to D-278; V-246 to D-278; R-247 to D-278; L-248 to D-278; F-249 to D-278; K-250 to D-278; R-251 to D-278; Q-252 to D-278; R-253 to D-278; E-254 to D-278; N-255 to D-278; A-256 to D-278; I-257 to D-278; Y-258 to D-278; S-259 to D-278; N-260 to D-278; D-261 to D-278; F-262 to D-278; D-263 to D-278; T-264 to D-278; Y-265 to D-278; I-266 to D-278; T-267 to D-278; F-268 to D-278; S-269 to D-278; G-270 to D-278; H-271 to D-278; L-272 to D-278; and I-273 to D-278 of SEQ ID NO: 58.

[0214] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0215] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 58: M-132 to D-277; M-132 to E-276; M-132 to A-275; M-132 to K-274; M-132 to I-273; M-132 to L-272; M-132 to H-271; M-132 to G-270; M-132 to S-269; M-132 to F-268; M-132 to T-267; M-132 to I-266; M-132 to Y-265; M-132 to T-264; M-132 to D-263; M-132 to F-262; M-132 to D-261; M-132 to N-260; M-132 to S-259; M-132 to Y-258; M-132 to I-257; M-132 to A-256; M-132 to N-255; M-132 to E-254; M-132 to R-253; M-132 to Q-252; M-132 to R-251; M-132 to K-250; M-132 to F-249; M-132 to L-

248; M-132 to R-247; M-132 to V-246; M-132 to W-245; M-132 to V-244; M-132 to R-243; M-132 to D-242; M-132 to G-241; M-132 to Y-240; M-132 to A-239; M-132 to L-238; M-132 to D-237; M-132 to L-236; M-132 to M-235; M-132 to V-234; M-132 to S-233; M-132 to Q-232; M-132 to S-231; M-132 to Q-230; M-132 to M-229; M-132 to I-228; M-132 to S-227; M-132 to R-226; M-132 to E-225; M-132 to S-224; M-132 to P-223; M-132 to Q-222; M-132 to A-221; M-132 to Y-220; M-132 to L-219; M-132 to I-218; M-132 to V-217; M-132 to A-216; M-132 to E-215; M-132 to K-214; M-132 to Q-213; M-132 to N-212; M-132 to H-211; M-132 to M-210; M-132 to I-209; M-132 to H-208; M-132 to V-207; M-132 to Y-206; M-132 to T-205; M-132 to E-204; M-132 to K-203; M-132 to Y-202; M-132 to N-201; M-132 to W-200; M-132 to S-199; M-132 to H-198; M-132 to V-197; M-132 to N-196; M-132 to L-195; M-132 to S-194; M-132 to F-193; M-132 to F-192; M-132 to Y-191; M-132 to I-190; M-132 to G-189; M-132 to R-188; M-132 to L-187; M-132 to P-186; M-132 to A-185; M-132 to A-184; M-132 to F-183; M-132 to Q-182; M-132 to G-181; M-132 to T-180; M-132 to A-179; M-132 to M-178; M-132 to D-177; M-132 to F-176; M-132 to C-175; M-132 to G-174; M-132 to D-173; M-132 to L-172; M-132 to N-171; M-132 to V-170; M-132 to F-169; M-132 to V-168; M-132 to R-167; M-132 to E-166; M-132 to F-165; M-132 to L-164; M-132 to L-163; M-132 to T-162; M-132 to Q-161; M-132 to F-160; M-132 to D-159; M-132 to E-158; M-132 to G-157; M-132 to S-156; M-132 to H-155; M-132 to L-154; M-132 to A-153; M-132 to T-152; M-132 to K-151; M-132 to R-150; M-132 to G-149; M-132 to V-148; M-132 to S-147; M-132 to F-146; M-132 to A-145; M-132 to F-144; M-132 to F-143; M-132 to R-142; M-132 to K-141; M-132 to Q-140; M-132 to C-139; and M-132 to P-138 of SEQ ID NO: 58.

[0216] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention.

Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0217] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 58, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0218] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0219] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 203071, where this portion excludes any integer of amino acid residues from 1 to about 272 amino acids from the amino terminus of the complete amino

acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 203071, or any integer of amino acid residues from 1 to about 272 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 203071. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0220] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0221] It has been discovered that this gene is expressed in umbilical vein, fetal heart, microvascular endothelial cells, and placenta.

[0222] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism, and cardiovascular disorders.

[0223] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and cardiovascular systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, vascular, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0224] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both

type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0225] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0226] More generally, the expression of this gene in tissues of the cardiovascular system and homology to the C1q family of proteins, including a TNF-related protein, indicate that polynucleotides and polypeptides corresponding to this gene, including

antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of cardiovascular disorders (e.g., atherosclerosis, restenosis, and/or as described herein under "Cardiovascular Disorders").

[0227] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 10

[0228] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329839), and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties.). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0229] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin.

The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0230] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 59 as residues Gly-16 to Pro-30, Pro-42 to Gly-56, Gly-62 to Gly-77, Glu-93 to Gly-104, Glu-109 to Glu-114, Pro-121 to Gly-134, Ser-157 to Arg-162, Glu-174 to Thr-182, and Ile-283 to Leu-289. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0231] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 59 as residues Pro-136 to Leu-289. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as

described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0232] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 59 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0233] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0234] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and

otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0235] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 59: G-137 to L-289; V-138 to L-289; C-139 to L-289; R-140 to L-289; C-141 to L-289; G-142 to L-289; S-143 to L-289; I-144 to L-289; V-145 to L-289; L-146 to L-289; K-147 to L-289; S-148 to L-289; A-149 to L-289; F-150 to L-289; S-151 to L-289; V-152 to L-289; G-153 to L-289; I-154 to L-289; T-155 to L-289; T-156 to L-289; S-157 to L-289; Y-158 to L-289; P-159 to L-289; E-160 to L-289; E-161 to L-289; R-162 to L-289; L-163 to L-289; P-164 to L-289; I-165 to L-289; I-166 to L-289; F-167 to L-289; N-168 to L-289; K-169 to L-289; V-170 to L-289; L-171 to L-289; F-172 to L-289; N-173 to L-289; E-174 to L-289; G-175 to L-289; E-176 to L-289; H-177 to L-289; Y-178 to L-289; N-179 to L-289; P-180 to L-289; A-181 to L-289; T-182 to L-289; G-183 to L-289; K-184 to L-289; F-185 to L-289; I-186 to L-289; C-187 to L-289; A-188 to L-289; F-189 to L-289; P-190 to L-289; G-191 to L-289; I-192 to L-289; Y-193 to L-289; Y-194 to L-289; F-195 to L-289; S-196 to L-289; Y-197 to L-289; D-198 to L-289; I-199 to L-289; T-200 to L-289; L-201 to L-289; A-202 to L-289; N-203 to L-289; K-204 to L-289; H-205 to L-289; L-206 to L-289; A-207 to L-289; I-208 to L-289; G-209 to L-289; L-210 to L-289; V-211 to L-289; H-212 to L-289; N-213 to L-289; G-214 to L-289; Q-215 to L-289; Y-216 to L-289; R-217 to L-289; I-218 to L-289; K-219 to L-289; T-220 to L-289; F-221 to L-289; D-222 to L-289; A-223 to L-289; N-224 to L-289; T-225 to L-289; G-226 to L-289; N-227 to L-289; H-228 to L-289; D-229 to L-289; V-230 to L-289; A-231 to L-289; S-232 to L-289; G-233 to L-289; S-234 to L-289; T-235 to L-289; V-236 to L-289; I-237 to L-289; Y-238 to L-289; L-239 to L-289; Q-240 to L-289; P-241 to L-289; E-242 to L-289; D-243 to L-289; E-244 to L-289; V-245 to L-289; W-246 to L-289; L-247 to L-289; E-248 to L-289; I-249 to L-289; F-250 to L-289; F-251 to L-289; T-252 to L-289; D-253 to L-289; Q-254 to L-289; N-255 to L-289; G-256 to L-289; L-257 to L-289; F-258 to L-289; S-259 to L-289; D-260 to L-289; P-261 to L-289; G-262 to L-289; W-263 to L-289; A-264 to L-289; D-265 to L-289; S-266 to L-289; L-267 to L-289; F-268

to L-289; S-269 to L-289; G-270 to L-289; F-271 to L-289; L-272 to L-289; L-273 to L-289; Y-274 to L-289; V-275 to L-289; D-276 to L-289; T-277 to L-289; D-278 to L-289; Y-279 to L-289; L-280 to L-289; D-281 to L-289; S-282 to L-289; I-283 to L-289; and S-284 to L-289 of SEQ ID NO: 59.

[0236] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0237] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 59: P-136 to E-288; P-136 to D-287; P-136 to D-286; P-136 to E-285; P-136 to S-284; P-136 to I-283; P-136 to S-282; P-136 to D-281; P-136 to L-280; P-136 to Y-279; P-136 to D-278; P-136 to T-277; P-136 to D-276; P-136 to V-275; P-136 to Y-274; P-136 to L-273; P-136 to L-272; P-136 to F-271; P-136 to G-270; P-136 to S-269; P-136 to F-268; P-136 to L-267; P-136 to S-266; P-136 to D-265; P-136 to A-264; P-136 to W-263; P-136 to G-262; P-136 to P-261; P-136 to D-260; P-136 to S-259; P-136 to F-258; P-136 to L-257; P-136 to G-256; P-136 to N-255; P-136 to Q-254; P-136 to D-253; P-136 to T-252; P-136 to F-251; P-136 to F-250; P-136 to I-249; P-136 to E-248; P-136 to L-247; P-136 to W-246; P-136 to V-245; P-136 to E-244; P-136 to D-243; P-136 to E-242; P-136 to P-241; P-136 to Q-240; P-136 to L-239; P-136 to Y-238; P-136 to I-237; P-136 to V-236; P-136 to T-235; P-136 to S-234; P-136 to G-233; P-136 to S-232; P-136 to A-231; P-136 to V-230; P-136 to D-229; P-136 to H-228; P-136 to N-227; P-136 to G-226; P-136 to T-225; P-136 to N-224; P-136 to A-223; P-136 to D-222; P-136 to F-221; P-136 to T-220; P-136 to K-219; P-136 to I-218; P-136 to R-217; P-136 to Y-216; P-136 to Q-215; P-136 to G-214; P-136 to N-213; P-136 to H-212; P-136 to V-211; P-136 to L-210;

P-136 to G-209; P-136 to I-208; P-136 to A-207; P-136 to L-206; P-136 to H-205; P-136 to K-204; P-136 to N-203; P-136 to A-202; P-136 to L-201; P-136 to T-200; P-136 to I-199; P-136 to D-198; P-136 to Y-197; P-136 to S-196; P-136 to F-195; P-136 to Y-194; P-136 to Y-193; P-136 to I-192; P-136 to G-191; P-136 to P-190; P-136 to F-189; P-136 to A-188; P-136 to C-187; P-136 to I-186; P-136 to F-185; P-136 to K-184; P-136 to G-183; P-136 to T-182; P-136 to A-181; P-136 to P-180; P-136 to N-179; P-136 to Y-178; P-136 to H-177; P-136 to E-176; P-136 to G-175; P-136 to E-174; P-136 to N-173; P-136 to F-172; P-136 to L-171; P-136 to V-170; P-136 to K-169; P-136 to N-168; P-136 to F-167; P-136 to I-166; P-136 to I-165; P-136 to P-164; P-136 to L-163; P-136 to R-162; P-136 to E-161; P-136 to E-160; P-136 to P-159; P-136 to Y-158; P-136 to S-157; P-136 to T-156; P-136 to T-155; P-136 to I-154; P-136 to G-153; P-136 to V-152; P-136 to S-151; P-136 to F-150; P-136 to A-149; P-136 to S-148; P-136 to K-147; P-136 to L-146; P-136 to V-145; P-136 to I-144; P-136 to S-143; and P-136 to G-142 of SEQ ID NO: 59.

[0238] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0239] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or

alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 59, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0240] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0241] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 209124, where this portion excludes any integer of amino acid residues from 1 to about 283 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 209124, or any integer of amino acid residues from 1 to about 283 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209124. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0242] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0243] It has been discovered that this gene is expressed in monocytes, skeletal muscle, and human schwanoma tissue.

[0244] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, as well as immunological and neurological disorders.

[0245] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunological disorders and neural or integumentary disorders, particularly neurofibroma. For a number of disorders of the above tissues or cells, particularly of the endocrine and of the peripheral and sympathetic nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, immune, neural, integumentary, extracellular matrix, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0246] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis,

microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0247] In addition, the expression of this gene in monocytes and similarity of this gene to other members of the C1q family of proteins, including a complement C1q-TNFalpha related protein (Swiss-Prot Accession Q9BXJ2), suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0248] More generally, the expression of this gene in schwanoma tissue and homology to a complement C1q-TNFalpha related protein (Swiss-Prot Accession Q9BXJ2) indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of neurological disorders, such as for example, those disclosed herein under "Neural Activity and Neurological Diseases".

[0249] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 11

[0250] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329836), and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0251] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression

of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0252] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 60 as residues: Arg-25 to Ser-31, Pro-39 to Gly-49, Pro-63 to Gly-97, Ala-105 to Asn-114, Thr-116 to Gly-133, Gly-144 to Ser-150, Lys-158 to Leu-165, Glu-176 to Ser-184, Ser-254 to Phe-260, and Ala-277 to Glu-284. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0253] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 60 as residues Pro-138 to Val-285. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention.

Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0254] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 60 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0255] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0256] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0257] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 60: G-139 to V-285; P-140 to V-285; C-141 to V-285; S-142 to V-285; C-143 to V-285; G-144 to V-285; S-145 to V-285; G-146 to V-285; H-147 to V-285; T-148 to V-285; K-149 to V-285; S-150 to V-285; A-151 to V-285; F-152 to V-285; S-153 to V-285; V-154 to V-285; A-155 to V-285; V-156 to V-285; T-157 to V-285; K-158 to V-285; S-159 to V-285; Y-160 to V-285; P-161 to V-285; R-162 to V-285; E-163 to V-285; R-164 to V-285; L-165 to V-285; P-166 to V-285; I-167 to V-285; K-168 to V-285; F-169 to V-285; D-170 to V-285; K-171 to V-285; I-172 to V-285; L-173 to V-285; M-174 to V-285; N-175 to V-285; E-176 to V-285; G-177 to V-285; G-178 to V-285; H-179 to V-285; Y-180 to V-285; N-181 to V-285; A-182 to V-285; S-183 to V-285; S-184 to V-285; G-185 to V-285; K-186 to V-285; F-187 to V-285; V-188 to V-285; C-189 to V-285; G-190 to V-285; V-191 to V-285; P-192 to V-285; G-193 to V-285; I-194 to V-285; Y-195 to V-285; Y-196 to V-285; F-197 to V-285; T-198 to V-285; Y-199 to V-285; D-200 to V-285; I-201 to V-285; T-202 to V-285; L-203 to V-285; A-204 to V-285; N-205 to V-285; K-206 to V-285; H-207 to V-285; L-208 to V-285; A-209 to V-285; I-210 to V-285; G-211 to V-285; L-212 to V-285; V-213 to V-285; H-214 to V-285; N-215 to V-285; G-216 to V-285; Q-217 to V-285; Y-218 to V-285; R-219 to V-285; I-220 to V-285; R-221 to V-285; T-222 to V-285; F-223 to V-285; D-224 to V-285; A-225 to V-285; N-226 to V-285; T-227 to V-285; G-228 to V-285; N-229 to V-285; H-230 to V-285; D-231 to V-285; V-232 to V-285; A-233 to V-285; S-234 to V-285; G-235 to V-285; S-236 to V-285; T-237 to V-285; I-238 to V-285; L-239 to V-285; A-240 to V-285; L-241 to V-285; K-242 to V-285; Q-243 to V-285; G-244 to V-285; D-245 to V-285; E-246 to V-285; V-247 to V-285; W-248 to V-285; L-249 to V-285; Q-250 to V-285; I-251 to V-285; F-252 to V-285; Y-253 to V-285; S-254 to V-285; E-255 to V-285; Q-256 to V-285; N-257 to V-285; G-258 to V-285; L-259 to V-285; F-260 to V-285; Y-261 to V-285; D-262 to V-285; P-263 to V-285; Y-264 to V-285; W-265 to V-285; T-266 to V-285; D-267 to V-285; S-268 to V-285; L-269 to V-285; F-270 to V-285; T-271 to V-285; G-272 to V-285; F-273 to V-285; L-274 to V-285; I-275 to V-285; Y-276 to V-285; A-277 to V-285; D-278 to V-285; Q-279 to V-285; and D-280 to V-285 of SEQ ID NO: 60.

[0258] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0259] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 60: P-138 to E-284; P-138 to N-283; P-138 to P-282; P-138 to D-281; P-138 to D-280; P-138 to Q-279; P-138 to D-278; P-138 to A-277; P-138 to Y-276; P-138 to I-275; P-138 to L-274; P-138 to F-273; P-138 to G-272; P-138 to T-271; P-138 to F-270; P-138 to L-269; P-138 to S-268; P-138 to D-267; P-138 to T-266; P-138 to W-265; P-138 to Y-264; P-138 to P-263; P-138 to D-262; P-138 to Y-261; P-138 to F-260; P-138 to L-259; P-138 to G-258; P-138 to N-257; P-138 to Q-256; P-138 to E-255; P-138 to S-254; P-138 to Y-253; P-138 to F-252; P-138 to I-251; P-138 to Q-250; P-138 to L-249; P-138 to W-248; P-138 to V-247; P-138 to E-246; P-138 to D-245; P-138 to G-244; P-138 to Q-243; P-138 to K-242; P-138 to L-241; P-138 to A-240; P-138 to L-239; P-138 to I-238; P-138 to T-237; P-138 to S-236; P-138 to G-235; P-138 to S-234; P-138 to A-233; P-138 to V-232; P-138 to D-231; P-138 to H-230; P-138 to N-229; P-138 to G-228; P-138 to T-227; P-138 to N-226; P-138 to A-225; P-138 to D-224; P-138 to F-223; P-138 to T-222; P-138 to R-221; P-138 to I-220; P-138 to R-219; P-138 to Y-218; P-138 to Q-217; P-138 to G-216; P-138 to N-215; P-138 to H-214; P-138 to V-213; P-138 to L-212; P-138 to G-211; P-138 to I-210; P-138 to A-209; P-138 to L-208; P-138 to H-207; P-138 to K-206; P-138 to N-205; P-138 to A-204; P-138 to L-203; P-138 to T-202; P-138 to I-201; P-138 to D-200; P-138 to Y-199; P-138 to T-198; P-138 to F-197; P-138 to Y-196; P-138 to Y-195; P-138 to I-194; P-138 to G-193; P-138 to P-192; P-138 to V-191; P-138 to G-190; P-138 to C-189; P-138 to V-188; P-138 to F-187; P-138 to K-186; P-138 to G-185; P-138 to S-

184; P-138 to S-183; P-138 to A-182; P-138 to N-181; P-138 to Y-180; P-138 to H-179; P-138 to G-178; P-138 to G-177; P-138 to E-176; P-138 to N-175; P-138 to M-174; P-138 to L-173; P-138 to I-172; P-138 to K-171; P-138 to D-170; P-138 to F-169; P-138 to K-168; P-138 to I-167; P-138 to P-166; P-138 to L-165; P-138 to R-164; P-138 to E-163; P-138 to R-162; P-138 to P-161; P-138 to Y-160; P-138 to S-159; P-138 to K-158; P-138 to T-157; P-138 to V-156; P-138 to A-155; P-138 to V-154; P-138 to S-153; P-138 to F-152; P-138 to A-151; P-138 to S-150; P-138 to K-149; P-138 to T-148; P-138 to H-147; P-138 to G-146; P-138 to S-145; and P-138 to G-144 of SEQ ID NO: 60.

[0260] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0261] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 60, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including

fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0262] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0263] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, where this portion excludes any integer of amino acid residues from 1 to about 279 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, or any integer of amino acid residues from 1 to about 279 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-3696. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0264] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0265] Translation products of this gene stimulate glucose transport in adipocytes.

[0266] It has been discovered that this gene is expressed in small intestine, as well as fetal brain and glioblastoma tissues.

[0267] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to:

diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, and neurological disorders.

[0268] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, intestinal, neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0269] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and

disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0270] In addition, the similarity of this gene to other members of the C1q family of proteins, including a TNF-related protein, suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0271] More generally, the expression of this gene in neural tissues and homology to the C1q family of proteins indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of neurological disorders, such as for example, those disclosed herein under "Neural Activity and Neurological Diseases".

[0272] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 12

[0273] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329838), and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties.).

ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0274] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0275] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, or all three of the immunogenic epitopes shown in SEQ ID NO: 61 as residues: Ala-9 to Gln-16, Asp-77 to Gln-87, and Asp-107 to Lys-119. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0276] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 61 as residues Thr-1 to Leu-146. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0277] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 61 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0278] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein

of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0279] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0280] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 61: R-2 to L-146; S-3 to L-146; L-4 to L-146; V-5 to L-146; G-6 to L-146; S-7 to L-146; D-8 to L-146; A-9 to L-146; G-10 to L-146; P-11 to L-146; G-12 to L-146; P-13 to L-146; R-14 to L-146; H-15 to L-146; Q-16 to L-146; P-17 to L-146; L-18 to L-146; A-19 to L-146; F-20 to L-146; D-21 to L-146; T-22 to L-146; E-23 to L-146; F-24 to L-146; V-25 to L-146; N-26 to L-146; I-27 to L-146; G-28 to L-146; G-29 to L-146; D-30 to L-146; F-31 to L-146; D-32 to L-146; A-33 to L-146; A-34 to L-146; A-35 to L-146; G-36 to L-146; V-37 to L-146; F-38 to L-146; R-39 to L-146; C-40 to L-146; R-41 to L-146; L-42 to L-146; P-43 to L-146; G-44 to L-146; A-45 to L-146; Y-46 to

L-146; F-47 to L-146; F-48 to L-146; S-49 to L-146; F-50 to L-146; T-51 to L-146; L-52 to L-146; G-53 to L-146; K-54 to L-146; L-55 to L-146; P-56 to L-146; R-57 to L-146; K-58 to L-146; T-59 to L-146; L-60 to L-146; S-61 to L-146; V-62 to L-146; K-63 to L-146; L-64 to L-146; M-65 to L-146; K-66 to L-146; N-67 to L-146; R-68 to L-146; D-69 to L-146; E-70 to L-146; V-71 to L-146; Q-72 to L-146; A-73 to L-146; M-74 to L-146; I-75 to L-146; Y-76 to L-146; D-77 to L-146; D-78 to L-146; G-79 to L-146; A-80 to L-146; S-81 to L-146; R-82 to L-146; R-83 to L-146; R-84 to L-146; E-85 to L-146; M-86 to L-146; Q-87 to L-146; S-88 to L-146; Q-89 to L-146; S-90 to L-146; V-91 to L-146; M-92 to L-146; L-93 to L-146; A-94 to L-146; L-95 to L-146; R-96 to L-146; R-97 to L-146; G-98 to L-146; D-99 to L-146; A-100 to L-146; V-101 to L-146; W-102 to L-146; L-103 to L-146; L-104 to L-146; S-105 to L-146; H-106 to L-146; D-107 to L-146; H-108 to L-146; D-109 to L-146; G-110 to L-146; Y-111 to L-146; G-112 to L-146; A-113 to L-146; Y-114 to L-146; S-115 to L-146; N-116 to L-146; H-117 to L-146; G-118 to L-146; K-119 to L-146; Y-120 to L-146; I-121 to L-146; T-122 to L-146; F-123 to L-146; S-124 to L-146; G-125 to L-146; F-126 to L-146; L-127 to L-146; V-128 to L-146; Y-129 to L-146; P-130 to L-146; D-131 to L-146; L-132 to L-146; A-133 to L-146; P-134 to L-146; A-135 to L-146; A-136 to L-146; P-137 to L-146; P-138 to L-146; G-139 to L-146; L-140 to L-146; and G-141 to L-146 of SEQ ID NO: 61.

[0281] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0282] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 61: T-1 to L-145; T-1 to E-144; T-1 to S-143; T-1 to A-142; T-1 to G-141;

T-1 to L-140; T-1 to G-139; T-1 to P-138; T-1 to P-137; T-1 to A-136; T-1 to A-135; T-1 to P-134; T-1 to A-133; T-1 to L-132; T-1 to D-131; T-1 to P-130; T-1 to Y-129; T-1 to V-128; T-1 to L-127; T-1 to F-126; T-1 to G-125; T-1 to S-124; T-1 to F-123; T-1 to T-122; T-1 to I-121; T-1 to Y-120; T-1 to K-119; T-1 to G-118; T-1 to H-117; T-1 to N-116; T-1 to S-115; T-1 to Y-114; T-1 to A-113; T-1 to G-112; T-1 to Y-111; T-1 to G-110; T-1 to D-109; T-1 to H-108; T-1 to D-107; T-1 to H-106; T-1 to S-105; T-1 to L-104; T-1 to L-103; T-1 to W-102; T-1 to V-101; T-1 to A-100; T-1 to D-99; T-1 to G-98; T-1 to R-97; T-1 to R-96; T-1 to L-95; T-1 to A-94; T-1 to L-93; T-1 to M-92; T-1 to V-91; T-1 to S-90; T-1 to Q-89; T-1 to S-88; T-1 to Q-87; T-1 to M-86; T-1 to E-85; T-1 to R-84; T-1 to R-83; T-1 to R-82; T-1 to S-81; T-1 to A-80; T-1 to G-79; T-1 to D-78; T-1 to D-77; T-1 to Y-76; T-1 to I-75; T-1 to M-74; T-1 to A-73; T-1 to Q-72; T-1 to V-71; T-1 to E-70; T-1 to D-69; T-1 to R-68; T-1 to N-67; T-1 to K-66; T-1 to M-65; T-1 to L-64; T-1 to K-63; T-1 to V-62; T-1 to S-61; T-1 to L-60; T-1 to T-59; T-1 to K-58; T-1 to R-57; T-1 to P-56; T-1 to L-55; T-1 to K-54; T-1 to G-53; T-1 to L-52; T-1 to T-51; T-1 to F-50; T-1 to S-49; T-1 to F-48; T-1 to F-47; T-1 to Y-46; T-1 to A-45; T-1 to G-44; T-1 to P-43; T-1 to L-42; T-1 to R-41; T-1 to C-40; T-1 to R-39; T-1 to F-38; T-1 to V-37; T-1 to G-36; T-1 to A-35; T-1 to A-34; T-1 to A-33; T-1 to D-32; T-1 to F-31; T-1 to D-30; T-1 to G-29; T-1 to G-28; T-1 to I-27; T-1 to N-26; T-1 to V-25; T-1 to F-24; T-1 to E-23; T-1 to T-22; T-1 to D-21; T-1 to F-20; T-1 to A-19; T-1 to L-18; T-1 to P-17; T-1 to Q-16; T-1 to H-15; T-1 to R-14; T-1 to P-13; T-1 to G-12; T-1 to P-11; T-1 to G-10; T-1 to A-9; T-1 to D-8; and T-1 to S-7 of SEQ ID NO: 61.

[0283] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0284] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 61, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0285] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, where this portion excludes any integer of amino acid residues from 1 to about 140 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3696, or any integer of amino acid residues from 1 to about 140 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-3696. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0286] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0287] It has been discovered that this gene is expressed in neural tissues (e.g., striatum, pituitary, and glioblastoma).

[0288] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, as well as neurological disorders.

[0289] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0290] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below),

endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0291] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0292] More generally, the expression of this gene in neural tissues and homology to C1q family proteins indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of neurodegenerative disorders, such as those described herein under "Neural Activity and Neurological Diseases".

[0293] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 13

[0294] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including a TNF-related protein (see, e.g. Genbank Accession No. AF329836), and ACRP30, an adipocyte complement-related

protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties.). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0295] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding

agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0296] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 62 as residues: Pro-45 to Gly-52, Asn-83 to Gly-97, Pro-105 to Thr-117, Arg-133 to Ile-138, Met-147 to Phe-158, Arg-184 to Ala-189. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0297] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 62 as residues Lys-108 to Ala-251. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0298] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 62 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention.

Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0299] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0300] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0301] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 62: G-109 to A-251; E-110 to A-251; S-111 to A-251; G-112 to A-251; D-113 to A-251; Y-114 to A-251; K-115 to A-251; A-116 to A-251; T-117 to A-251; Q-118 to A-251; K-119 to A-251; I-120 to A-251; A-121 to A-251; F-122 to A-251; S-123 to A-251; A-124 to A-251; T-125 to A-251; R-126 to A-251; T-127 to A-

251; I-128 to A-251; N-129 to A-251; V-130 to A-251; P-131 to A-251; L-132 to A-251; R-133 to A-251; R-134 to A-251; D-135 to A-251; Q-136 to A-251; T-137 to A-251; I-138 to A-251; R-139 to A-251; F-140 to A-251; D-141 to A-251; H-142 to A-251; V-143 to A-251; I-144 to A-251; T-145 to A-251; N-146 to A-251; M-147 to A-251; N-148 to A-251; N-149 to A-251; N-150 to A-251; Y-151 to A-251; E-152 to A-251; P-153 to A-251; R-154 to A-251; S-155 to A-251; G-156 to A-251; K-157 to A-251; F-158 to A-251; T-159 to A-251; C-160 to A-251; K-161 to A-251; V-162 to A-251; P-163 to A-251; G-164 to A-251; L-165 to A-251; Y-166 to A-251; Y-167 to A-251; F-168 to A-251; T-169 to A-251; Y-170 to A-251; H-171 to A-251; A-172 to A-251; S-173 to A-251; S-174 to A-251; R-175 to A-251; G-176 to A-251; N-177 to A-251; L-178 to A-251; C-179 to A-251; V-180 to A-251; N-181 to A-251; L-182 to A-251; M-183 to A-251; R-184 to A-251; G-185 to A-251; R-186 to A-251; E-187 to A-251; R-188 to A-251; A-189 to A-251; Q-190 to A-251; K-191 to A-251; V-192 to A-251; V-193 to A-251; T-194 to A-251; F-195 to A-251; C-196 to A-251; D-197 to A-251; Y-198 to A-251; A-199 to A-251; Y-200 to A-251; N-201 to A-251; T-202 to A-251; F-203 to A-251; Q-204 to A-251; V-205 to A-251; T-206 to A-251; T-207 to A-251; G-208 to A-251; G-209 to A-251; M-210 to A-251; V-211 to A-251; L-212 to A-251; K-213 to A-251; L-214 to A-251; E-215 to A-251; Q-216 to A-251; G-217 to A-251; E-218 to A-251; N-219 to A-251; V-220 to A-251; F-221 to A-251; L-222 to A-251; Q-223 to A-251; A-224 to A-251; T-225 to A-251; D-226 to A-251; K-227 to A-251; N-228 to A-251; S-229 to A-251; L-230 to A-251; L-231 to A-251; G-232 to A-251; M-233 to A-251; E-234 to A-251; G-235 to A-251; A-236 to A-251; N-237 to A-251; S-238 to A-251; I-239 to A-251; F-240 to A-251; S-241 to A-251; G-242 to A-251; F-243 to A-251; L-244 to A-251; L-245 to A-251; and F-246 to A-251 of SEQ ID NO: 62.

[0302] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention.

Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0303] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 62: K-108 to E-250; K-108 to M-249; K-108 to D-248; K-108 to P-247; K-108 to F-246; K-108 to L-245; K-108 to L-244; K-108 to F-243; K-108 to G-242; K-108 to S-241; K-108 to F-240; K-108 to I-239; K-108 to S-238; K-108 to N-237; K-108 to A-236; K-108 to G-235; K-108 to E-234; K-108 to M-233; K-108 to G-232; K-108 to L-231; K-108 to L-230; K-108 to S-229; K-108 to N-228; K-108 to K-227; K-108 to D-226; K-108 to T-225; K-108 to A-224; K-108 to Q-223; K-108 to L-222; K-108 to F-221; K-108 to V-220; K-108 to N-219; K-108 to E-218; K-108 to G-217; K-108 to Q-216; K-108 to E-215; K-108 to L-214; K-108 to K-213; K-108 to L-212; K-108 to V-211; K-108 to M-210; K-108 to G-209; K-108 to G-208; K-108 to T-207; K-108 to T-206; K-108 to V-205; K-108 to Q-204; K-108 to F-203; K-108 to T-202; K-108 to N-201; K-108 to Y-200; K-108 to A-199; K-108 to Y-198; K-108 to D-197; K-108 to C-196; K-108 to F-195; K-108 to T-194; K-108 to V-193; K-108 to V-192; K-108 to K-191; K-108 to Q-190; K-108 to A-189; K-108 to R-188; K-108 to E-187; K-108 to R-186; K-108 to G-185; K-108 to R-184; K-108 to M-183; K-108 to L-182; K-108 to N-181; K-108 to V-180; K-108 to C-179; K-108 to L-178; K-108 to N-177; K-108 to G-176; K-108 to R-175; K-108 to S-174; K-108 to S-173; K-108 to A-172; K-108 to H-171; K-108 to Y-170; K-108 to T-169; K-108 to F-168; K-108 to Y-167; K-108 to Y-166; K-108 to L-165; K-108 to G-164; K-108 to P-163; K-108 to V-162; K-108 to K-161; K-108 to C-160; K-108 to T-159; K-108 to F-158; K-108 to K-157; K-108 to G-156; K-108 to S-155; K-108 to R-154; K-108 to P-153; K-108 to E-152; K-108 to Y-151; K-108 to N-150; K-108 to N-149; K-108 to N-148; K-108 to M-147; K-108 to N-146; K-108 to T-145; K-108 to I-144; K-108 to V-143; K-108 to H-142; K-108 to D-141; K-108 to F-140; K-108 to R-139; K-108 to I-138; K-108 to T-137; K-108 to Q-136; K-108 to D-135; K-108 to R-134; K-108 to R-133; K-108 to L-132; K-108 to P-131; K-108 to V-130; K-108 to N-129; K-108 to I-128; K-108 to T-127; K-108 to R-126; K-108 to T-125; K-108 to A-124; K-108 to S-123; K-108 to F-122; K-108 to A-121; K-108 to I-120; K-108 to K-119; K-108 to Q-118; K-108 to T-117; K-108 to A-116; K-108 to K-115; and K-108 to Y-114 of SEQ ID NO: 62.

[0304] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0305] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 62, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0306] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, where this portion excludes any integer of amino acid residues from 1 to about 245 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, or any integer of amino acid residues from 1 to about 245

amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0307] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0308] It has been discovered that this gene is expressed in tissues of the immune system, including primary dendritic cells, monocytes, bone marrow, spleen, and T cell lymphoma, as well as the colon.

[0309] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes and diseases and/or disorders involving dysfunctional fatty acid metabolism, as well as immunological and gastrointestinal disorders.

[0310] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, immune, and gastrointestinal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, immune, gastrointestinal, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0311] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both

type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0312] In addition, the expression of this gene in immune system cells and tissues, and similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0313] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 14

[0314] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0315] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of

expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0316] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 63 as residues: Pro-15 to Thr-23, Arg-36 to Thr-50, Arg-52 to Leu-80, Gly-84 to Trp-90, Asp-93 to Lys-105, Glu-132 to Arg-146, Ser-151 to Thr-159, Gly-163 to Gln-169, Asn-175 to Gly-180, Asp-193 to Glu-203, Leu-205 to Lys-210, Lys-263 to Tyr-268, Gln-276 to Ser-282, Glu-293 to Asn-303, Asn-317 to Ile-327, Ile-331 to Ile-338, Arg-351 to Asp-357, Pro-491 to Arg-496, Lys-515 to Asp-529, Gln-548 to Gly-557, Val-568 to Val-575, Leu-592 to Gln-600, Arg-671 to Gly-679, Pro-706 to Gly-735, Thr-738 to Arg-744, Pro-746 to Gly-762, Gly-789 to Gly-795, Pro-809 to Pro-815, Thr-835 to Ser-841, Gly-856 to Ser-862, and Glu-910 to Gly-919. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0317] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 63 as residues Pro-816 to Leu-975. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these

polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0318] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 63 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0319] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0320] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such

immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0321] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 63: V-817 to L-975; A-818 to L-975; S-819 to L-975; P-820 to L-975; G-821 to L-975; A-822 to L-975; P-823 to L-975; V-824 to L-975; P-825 to L-975; S-826 to L-975; L-827 to L-975; V-828 to L-975; S-829 to L-975; F-830 to L-975; S-831 to L-975; A-832 to L-975; G-833 to L-975; L-834 to L-975; T-835 to L-975; Q-836 to L-975; K-837 to L-975; P-838 to L-975; F-839 to L-975; P-840 to L-975; S-841 to L-975; D-842 to L-975; G-843 to L-975; G-844 to L-975; V-845 to L-975; V-846 to L-975; L-847 to L-975; F-848 to L-975; N-849 to L-975; K-850 to L-975; V-851 to L-975; L-852 to L-975; V-853 to L-975; N-854 to L-975; D-855 to L-975; G-856 to L-975; D-857 to L-975; V-858 to L-975; Y-859 to L-975; N-860 to L-975; P-861 to L-975; S-862 to L-975; T-863 to L-975; G-864 to L-975; V-865 to L-975; F-866 to L-975; T-867 to L-975; A-868 to L-975; P-869 to L-975; Y-870 to L-975; D-871 to L-975; G-872 to L-975; R-873 to L-975; Y-874 to L-975; L-875 to L-975; I-876 to L-975; T-877 to L-975; A-878 to L-975; T-879 to L-975; L-880 to L-975; T-881 to L-975; P-882 to L-975; E-883 to L-975; R-884 to L-975; D-885 to L-975; A-886 to L-975; Y-887 to L-975; V-888 to L-975; E-889 to L-975; A-890 to L-975; V-891 to L-975; L-892 to L-975; S-893 to L-975; V-894 to L-975; S-895 to L-975; N-896 to L-975; A-897 to L-975; S-898 to L-975; V-899 to L-975; A-900 to L-975; Q-901 to L-975; L-902 to L-975; H-903 to L-975; T-904 to L-975; A-905 to L-975; G-906 to L-975; Y-907 to L-975; R-908 to L-975; R-909 to L-975; E-910 to L-975; F-911 to L-975; L-912 to L-975; E-913 to L-975; Y-914 to L-975; H-915 to L-975; R-916 to L-975; P-917 to L-975; P-918 to L-975; G-919 to L-975; A-920 to L-975; L-921 to L-975; H-922 to L-975; T-923 to L-975; C-924 to L-975; G-925 to L-975; G-926 to L-975; P-927 to L-975; G-928 to L-975; A-929 to L-975; F-930 to L-975; H-931 to L-975; L-932 to L-975; I-933 to L-975; V-934 to L-975; H-935 to L-975; L-936 to L-975; K-937 to L-975; A-938 to L-975; G-939 to L-975; D-940 to L-975; A-941 to L-975; V-942 to L-975;

N-943 to L-975; V-944 to L-975; V-945 to L-975; V-946 to L-975; T-947 to L-975; G-948 to L-975; G-949 to L-975; K-950 to L-975; L-951 to L-975; A-952 to L-975; H-953 to L-975; T-954 to L-975; D-955 to L-975; F-956 to L-975; D-957 to L-975; E-958 to L-975; M-959 to L-975; Y-960 to L-975; S-961 to L-975; T-962 to L-975; F-963 to L-975; S-964 to L-975; G-965 to L-975; V-966 to L-975; F-967 to L-975; L-968 to L-975; Y-969 to L-975; and P-970 to L-975 of SEQ ID NO: 63.

[0322] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0323] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 63: P-816 to H-974; P-816 to S-973; P-816 to L-972; P-816 to F-971; P-816 to P-970; P-816 to Y-969; P-816 to L-968; P-816 to F-967; P-816 to V-966; P-816 to G-965; P-816 to S-964; P-816 to F-963; P-816 to T-962; P-816 to S-961; P-816 to Y-960; P-816 to M-959; P-816 to E-958; P-816 to D-957; P-816 to F-956; P-816 to D-955; P-816 to T-954; P-816 to H-953; P-816 to A-952; P-816 to L-951; P-816 to K-950; P-816 to G-949; P-816 to G-948; P-816 to T-947; P-816 to V-946; P-816 to V-945; P-816 to V-944; P-816 to N-943; P-816 to V-942; P-816 to A-941; P-816 to D-940; P-816 to G-939; P-816 to A-938; P-816 to K-937; P-816 to L-936; P-816 to H-935; P-816 to V-934; P-816 to I-933; P-816 to L-932; P-816 to H-931; P-816 to F-930; P-816 to A-929; P-816 to G-928; P-816 to P-927; P-816 to G-926; P-816 to G-925; P-816 to C-924; P-816 to T-923; P-816 to H-922; P-816 to L-921; P-816 to A-920; P-816 to G-919; P-816 to P-918; P-816 to P-917; P-816 to R-916; P-816 to H-915; P-816 to Y-914; P-816 to E-913; P-816 to L-912; P-816 to F-911; P-816 to E-910; P-816 to R-909; P-816 to R-908; P-816 to Y-907; P-816 to G-

906; P-816 to A-905; P-816 to T-904; P-816 to H-903; P-816 to L-902; P-816 to Q-901; P-816 to A-900; P-816 to V-899; P-816 to S-898; P-816 to A-897; P-816 to N-896; P-816 to S-895; P-816 to V-894; P-816 to S-893; P-816 to L-892; P-816 to V-891; P-816 to A-890; P-816 to E-889; P-816 to V-888; P-816 to Y-887; P-816 to A-886; P-816 to D-885; P-816 to R-884; P-816 to E-883; P-816 to P-882; P-816 to T-881; P-816 to L-880; P-816 to T-879; P-816 to A-878; P-816 to T-877; P-816 to I-876; P-816 to L-875; P-816 to Y-874; P-816 to R-873; P-816 to G-872; P-816 to D-871; P-816 to Y-870; P-816 to P-869; P-816 to A-868; P-816 to T-867; P-816 to F-866; P-816 to V-865; P-816 to G-864; P-816 to T-863; P-816 to S-862; P-816 to P-861; P-816 to N-860; P-816 to Y-859; P-816 to V-858; P-816 to D-857; P-816 to G-856; P-816 to D-855; P-816 to N-854; P-816 to V-853; P-816 to L-852; P-816 to V-851; P-816 to K-850; P-816 to N-849; P-816 to F-848; P-816 to L-847; P-816 to V-846; P-816 to V-845; P-816 to G-844; P-816 to G-843; P-816 to D-842; P-816 to S-841; P-816 to P-840; P-816 to F-839; P-816 to P-838; P-816 to K-837; P-816 to Q-836; P-816 to T-835; P-816 to L-834; P-816 to G-833; P-816 to A-832; P-816 to S-831; P-816 to F-830; P-816 to S-829; P-816 to V-828; P-816 to L-827; P-816 to S-826; P-816 to P-825; P-816 to V-824; P-816 to P-823; and P-816 to A-822 of SEQ ID NO: 63.

[0324] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0325] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n

corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 63, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0326] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0327] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, where this portion excludes any integer of amino acid residues from 1 to about 969 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, or any integer of amino acid residues from 1 to about 969 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0328] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0329] Translation products of this gene stimulate glucose transport in adipocytes.

[0330] It has been discovered that this gene is expressed in primary dendritic cells, monocytes, and macrophages.

[0331] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, and immunological disorders.

[0332] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, immune, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0333] The structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus,

"IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0334] In addition, expression of this gene in primary dendritic cells, monocytes, and macrophages and the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0335] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 15

[0336] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0337] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by

increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0338] Preferred polypeptides of the present invention comprise, or alternatively consist of, one or both of the immunogenic epitopes shown in SEQ ID NO: 64 as residues: Asn-108 to Ser-118, and Ser-143 to Phe-150. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0339] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 64 as residues Pro-17 to Asp-158. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0340] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 64 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention.

Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0341] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0342] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0343] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 64: V-18 to D-158; H-19 to D-158; V-20 to D-158; Y-21 to D-158; P-22 to D-158; L-23 to D-158; P-24 to D-158; Q-25 to D-158; Q-26 to D-158; M-27 to D-158; R-28 to D-158; V-29 to D-158; A-30 to D-158; F-31 to D-158; S-32 to D-158; A-33 to D-158; A-34 to D-158; R-35 to D-158; T-36 to D-158; S-37 to D-158; N-38

to D-158; L-39 to D-158; A-40 to D-158; P-41 to D-158; G-42 to D-158; T-43 to D-158; L-44 to D-158; D-45 to D-158; Q-46 to D-158; P-47 to D-158; I-48 to D-158; V-49 to D-158; F-50 to D-158; D-51 to D-158; L-52 to D-158; L-53 to D-158; L-54 to D-158; N-55 to D-158; N-56 to D-158; L-57 to D-158; G-58 to D-158; E-59 to D-158; T-60 to D-158; F-61 to D-158; D-62 to D-158; L-63 to D-158; Q-64 to D-158; L-65 to D-158; G-66 to D-158; R-67 to D-158; F-68 to D-158; N-69 to D-158; C-70 to D-158; P-71 to D-158; V-72 to D-158; N-73 to D-158; G-74 to D-158; T-75 to D-158; Y-76 to D-158; V-77 to D-158; F-78 to D-158; I-79 to D-158; F-80 to D-158; H-81 to D-158; M-82 to D-158; L-83 to D-158; K-84 to D-158; L-85 to D-158; A-86 to D-158; V-87 to D-158; N-88 to D-158; V-89 to D-158; P-90 to D-158; L-91 to D-158; Y-92 to D-158; V-93 to D-158; N-94 to D-158; L-95 to D-158; M-96 to D-158; K-97 to D-158; N-98 to D-158; E-99 to D-158; E-100 to D-158; V-101 to D-158; L-102 to D-158; V-103 to D-158; S-104 to D-158; A-105 to D-158; Y-106 to D-158; A-107 to D-158; N-108 to D-158; D-109 to D-158; G-110 to D-158; A-111 to D-158; P-112 to D-158; D-113 to D-158; H-114 to D-158; E-115 to D-158; T-116 to D-158; A-117 to D-158; S-118 to D-158; N-119 to D-158; H-120 to D-158; A-121 to D-158; I-122 to D-158; L-123 to D-158; Q-124 to D-158; L-125 to D-158; F-126 to D-158; Q-127 to D-158; G-128 to D-158; D-129 to D-158; Q-130 to D-158; I-131 to D-158; W-132 to D-158; L-133 to D-158; R-134 to D-158; L-135 to D-158; H-136 to D-158; R-137 to D-158; G-138 to D-158; A-139 to D-158; I-140 to D-158; Y-141 to D-158; G-142 to D-158; S-143 to D-158; S-144 to D-158; W-145 to D-158; K-146 to D-158; Y-147 to D-158; S-148 to D-158; T-149 to D-158; F-150 to D-158; S-151 to D-158; G-152 to D-158; and Y-153 to D-158 of SEQ ID NO: 64.

[0344] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0345] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 64: P-17 to Q-157; P-17 to Y-156; P-17 to L-155; P-17 to L-154; P-17 to Y-153; P-17 to G-152; P-17 to S-151; P-17 to F-150; P-17 to T-149; P-17 to S-148; P-17 to Y-147; P-17 to K-146; P-17 to W-145; P-17 to S-144; P-17 to S-143; P-17 to G-142; P-17 to Y-141; P-17 to I-140; P-17 to A-139; P-17 to G-138; P-17 to R-137; P-17 to H-136; P-17 to L-135; P-17 to R-134; P-17 to L-133; P-17 to W-132; P-17 to I-131; P-17 to Q-130; P-17 to D-129; P-17 to G-128; P-17 to Q-127; P-17 to F-126; P-17 to L-125; P-17 to Q-124; P-17 to L-123; P-17 to I-122; P-17 to A-121; P-17 to H-120; P-17 to N-119; P-17 to S-118; P-17 to A-117; P-17 to T-116; P-17 to E-115; P-17 to H-114; P-17 to D-113; P-17 to P-112; P-17 to A-111; P-17 to G-110; P-17 to D-109; P-17 to N-108; P-17 to A-107; P-17 to Y-106; P-17 to A-105; P-17 to S-104; P-17 to V-103; P-17 to L-102; P-17 to V-101; P-17 to E-100; P-17 to E-99; P-17 to N-98; P-17 to K-97; P-17 to M-96; P-17 to L-95; P-17 to N-94; P-17 to V-93; P-17 to Y-92; P-17 to L-91; P-17 to P-90; P-17 to V-89; P-17 to N-88; P-17 to V-87; P-17 to A-86; P-17 to L-85; P-17 to K-84; P-17 to L-83; P-17 to M-82; P-17 to H-81; P-17 to F-80; P-17 to I-79; P-17 to F-78; P-17 to V-77; P-17 to Y-76; P-17 to T-75; P-17 to G-74; P-17 to N-73; P-17 to V-72; P-17 to P-71; P-17 to C-70; P-17 to N-69; P-17 to F-68; P-17 to R-67; P-17 to G-66; P-17 to L-65; P-17 to Q-64; P-17 to L-63; P-17 to D-62; P-17 to F-61; P-17 to T-60; P-17 to E-59; P-17 to G-58; P-17 to L-57; P-17 to N-56; P-17 to N-55; P-17 to L-54; P-17 to L-53; P-17 to L-52; P-17 to D-51; P-17 to F-50; P-17 to V-49; P-17 to I-48; P-17 to P-47; P-17 to Q-46; P-17 to D-45; P-17 to L-44; P-17 to T-43; P-17 to G-42; P-17 to P-41; P-17 to A-40; P-17 to L-39; P-17 to N-38; P-17 to S-37; P-17 to T-36; P-17 to R-35; P-17 to A-34; P-17 to A-33; P-17 to S-32; P-17 to F-31; P-17 to A-30; P-17 to V-29; P-17 to R-28; P-17 to M-27; P-17 to Q-26; P-17 to Q-25; P-17 to P-24; and P-17 to L-23 of SEQ ID NO: 64.

[0346] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the

complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0347] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 64, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0348] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0349] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in

ATCC Deposit Nos. PTA-2574 and PTA-2575, where this portion excludes any integer of amino acid residues from 1 to about 152 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, or any integer of amino acid residues from 1 to about 152 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0350] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0351] It has been discovered that this gene is expressed in skeletal muscle as well as neural tissues, including infant brain and neuron-derived libraries.

[0352] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, and neurological disorders.

[0353] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., muscle, adipose, neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0354] The expression of this gene in skeletal muscle and structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein

under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0355] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0356] More generally, the expression of this gene in neural tissues and homology to the C1q family of proteins indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of neurological disorders, such as for example, those disclosed herein under "Neural Activity and Neurological Diseases".

[0357] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 16

[0358] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including alpha 1 (VIII) collagen (e.g., see Genbank Accession No. X57527), a matrix protein involved in tissue remodeling such as in injured arteries and atherosclerotic plaques; and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0359] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares

significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0360] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 65 as residues: Leu-6 to Gln-16, Pro-56 to His-63, Pro-77 to Gly-90, Arg-98 to Phe-106, Lys-115 to Met-120, Pro-141 to Leu-147, Pro-153 to Gln-159, Pro-191 to Glu-196, Pro-217 to Met-225, Pro-234 to Gly-239, Gln-283 to Gly-290, Pro-321 to Lys-328, Pro-349 to Gly-356, Pro-422 to Gly-430, Thr-438 to Leu-446, Tyr-462 to Pro-472, Tyr-501 to Thr-511, and Thr-549 to Phe-557. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0361] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 65 as residues Lys-466 to Met-605. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0362] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 65 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0363] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0364] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to

induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0365] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 65: G-467 to M-605; K-468 to M-605; N-469 to M-605; G-470 to M-605; G-471 to M-605; P-472 to M-605; A-473 to M-605; Y-474 to M-605; E-475 to M-605; M-476 to M-605; P-477 to M-605; A-478 to M-605; F-479 to M-605; T-480 to M-605; A-481 to M-605; E-482 to M-605; L-483 to M-605; T-484 to M-605; A-485 to M-605; P-486 to M-605; F-487 to M-605; P-488 to M-605; P-489 to M-605; V-490 to M-605; G-491 to M-605; A-492 to M-605; P-493 to M-605; V-494 to M-605; K-495 to M-605; F-496 to M-605; N-497 to M-605; K-498 to M-605; L-499 to M-605; L-500 to M-605; Y-501 to M-605; N-502 to M-605; G-503 to M-605; R-504 to M-605; Q-505 to M-605; N-506 to M-605; Y-507 to M-605; N-508 to M-605; P-509 to M-605; Q-510 to M-605; T-511 to M-605; G-512 to M-605; I-513 to M-605; F-514 to M-605; T-515 to M-605; C-516 to M-605; E-517 to M-605; V-518 to M-605; P-519 to M-605; G-520 to M-605; V-521 to M-605; Y-522 to M-605; Y-523 to M-605; F-524 to M-605; A-525 to M-605; Y-526 to M-605; H-527 to M-605; V-528 to M-605; H-529 to M-605; C-530 to M-605; K-531 to M-605; G-532 to M-605; G-533 to M-605; N-534 to M-605; V-535 to M-605; W-536 to M-605; V-537 to M-605; A-538 to M-605; L-539 to M-605; F-540 to M-605; K-541 to M-605; N-542 to M-605; N-543 to M-605; E-544 to M-605; P-545 to M-605; V-546 to M-605; M-547 to M-605; Y-548 to M-605; T-549 to M-605; Y-550 to M-605; D-551 to M-605; E-552 to M-605; Y-553 to M-605; K-554 to M-605; K-555 to M-605; G-556 to M-605; F-557 to M-605; L-558 to M-605; D-559 to M-605; Q-560 to M-605; A-561 to M-605; S-562 to M-605; G-563 to M-605; S-564 to M-605; A-565 to M-605; V-

566 to M-605; L-567 to M-605; L-568 to M-605; L-569 to M-605; R-570 to M-605; P-571 to M-605; G-572 to M-605; D-573 to M-605; R-574 to M-605; V-575 to M-605; F-576 to M-605; L-577 to M-605; Q-578 to M-605; M-579 to M-605; P-580 to M-605; S-581 to M-605; E-582 to M-605; Q-583 to M-605; A-584 to M-605; A-585 to M-605; G-586 to M-605; L-587 to M-605; Y-588 to M-605; A-589 to M-605; G-590 to M-605; Q-591 to M-605; Y-592 to M-605; V-593 to M-605; H-594 to M-605; S-595 to M-605; S-596 to M-605; F-597 to M-605; S-598 to M-605; G-599 to M-605; and Y-600 to M-605 of SEQ ID NO: 65.

[0366] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0367] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 65: K-466 to P-604; K-466 to Y-603; K-466 to L-602; K-466 to L-601; K-466 to Y-600; K-466 to G-599; K-466 to S-598; K-466 to F-597; K-466 to S-596; K-466 to S-595; K-466 to H-594; K-466 to V-593; K-466 to Y-592; K-466 to Q-591; K-466 to G-590; K-466 to A-589; K-466 to Y-588; K-466 to L-587; K-466 to G-586; K-466 to A-585; K-466 to A-584; K-466 to Q-583; K-466 to E-582; K-466 to S-581; K-466 to P-580; K-466 to M-579; K-466 to Q-578; K-466 to L-577; K-466 to F-576; K-466 to V-575; K-466 to R-574; K-466 to D-573; K-466 to G-572; K-466 to P-571; K-466 to R-570; K-466 to L-569; K-466 to L-568; K-466 to L-567; K-466 to V-566; K-466 to A-565; K-466 to S-564; K-466 to G-563; K-466 to S-562; K-466 to A-561; K-466 to Q-560; K-466 to D-559; K-466 to L-558; K-466 to F-557; K-466 to G-556; K-466 to K-555; K-466 to K-554; K-466 to Y-553; K-466 to E-552; K-466 to D-551; K-466 to Y-550; K-466 to T-549; K-466

to Y-548; K-466 to M-547; K-466 to V-546; K-466 to P-545; K-466 to E-544; K-466 to N-543; K-466 to N-542; K-466 to K-541; K-466 to F-540; K-466 to L-539; K-466 to A-538; K-466 to V-537; K-466 to W-536; K-466 to V-535; K-466 to N-534; K-466 to G-533; K-466 to G-532; K-466 to K-531; K-466 to C-530; K-466 to H-529; K-466 to V-528; K-466 to H-527; K-466 to Y-526; K-466 to A-525; K-466 to F-524; K-466 to Y-523; K-466 to Y-522; K-466 to V-521; K-466 to G-520; K-466 to P-519; K-466 to V-518; K-466 to E-517; K-466 to C-516; K-466 to T-515; K-466 to F-514; K-466 to I-513; K-466 to G-512; K-466 to T-511; K-466 to Q-510; K-466 to P-509; K-466 to N-508; K-466 to Y-507; K-466 to N-506; K-466 to Q-505; K-466 to R-504; K-466 to G-503; K-466 to N-502; K-466 to Y-501; K-466 to L-500; K-466 to L-499; K-466 to K-498; K-466 to N-497; K-466 to F-496; K-466 to K-495; K-466 to V-494; K-466 to P-493; K-466 to A-492; K-466 to G-491; K-466 to V-490; K-466 to P-489; K-466 to P-488; K-466 to F-487; K-466 to P-486; K-466 to A-485; K-466 to T-484; K-466 to L-483; K-466 to E-482; K-466 to A-481; K-466 to T-480; K-466 to F-479; K-466 to A-478; K-466 to P-477; K-466 to M-476; K-466 to E-475; K-466 to Y-474; K-466 to A-473; and K-466 to P-472 of SEQ ID NO: 65.

[0368] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0369] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention.

Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 65, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0370] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0371] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, where this portion excludes any integer of amino acid residues from 1 to about 599 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575, or any integer of amino acid residues from 1 to about 599 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Nos. PTA-2574 and PTA-2575. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0372] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0373] It has been discovered that this gene is expressed in adipocytes, smooth muscle, osteoblast stromal cells, osteoclastoma stromal cells, and bone marrow stromal cells.

[0374] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, as well as cardiovascular and bone disorders.

[0375] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, cardiovascular, and musculoskeletal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, muscle, bone, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0376] The expression of this gene in adipocytes and the structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma,

cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0377] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0378] Alternatively, the expression of this gene in smooth muscle tissue, and homology to alpha 1 (VIII) collagen indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of cardiovacular disorders, such as for example, atherosclerosis, restenosis, and/or those disclosed herein under "Cardiovascular Disorders".

[0379] Furthermore, the expression of this gene in a number of bone-related tissues and homology to alpha 1 (VIII) collagen indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis,

prevention, and/or treatment of bone and joint disorders, including osteoporosis, arthritis, and cancers of bone tissue, such as described under "Hyperproliferative Disorders" herein.

[0380] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 17

[0381] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including alpha 2 (VIII) collagen (e.g., see Genbank Accession No. AAA62822), a matrix protein involved in tissue remodeling such as in injured arteries and atherosclerotic plaques; and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0382] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin.

The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0383] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, or all four of the immunogenic epitopes shown in SEQ ID NO: 66 as residues: Pro-6 to Gly-22, Arg-87 to Pro-98, Asp-140 to Tyr-146, Pro-169 to Asn-174. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0384] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 66 as residues Phe-55 to Thr-194. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which

hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0385] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 66 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0386] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0387] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic

activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0388] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 66: G-56 to T-194; L-57 to T-194; G-58 to T-194; E-59 to T-194; L-60 to T-194; S-61 to T-194; A-62 to T-194; H-63 to T-194; A-64 to T-194; T-65 to T-194; P-66 to T-194; A-67 to T-194; F-68 to T-194; T-69 to T-194; A-70 to T-194; V-71 to T-194; L-72 to T-194; T-73 to T-194; S-74 to T-194; P-75 to T-194; F-76 to T-194; P-77 to T-194; A-78 to T-194; S-79 to T-194; G-80 to T-194; M-81 to T-194; P-82 to T-194; V-83 to T-194; K-84 to T-194; F-85 to T-194; D-86 to T-194; R-87 to T-194; T-88 to T-194; L-89 to T-194; Y-90 to T-194; N-91 to T-194; G-92 to T-194; H-93 to T-194; S-94 to T-194; G-95 to T-194; Y-96 to T-194; N-97 to T-194; P-98 to T-194; A-99 to T-194; T-100 to T-194; G-101 to T-194; I-102 to T-194; F-103 to T-194; T-104 to T-194; C-105 to T-194; P-106 to T-194; V-107 to T-194; G-108 to T-194; G-109 to T-194; V-110 to T-194; Y-111 to T-194; Y-112 to T-194; F-113 to T-194; A-114 to T-194; Y-115 to T-194; H-116 to T-194; V-117 to T-194; H-118 to T-194; V-119 to T-194; K-120 to T-194; G-121 to T-194; T-122 to T-194; N-123 to T-194; V-124 to T-194; W-125 to T-194; V-126 to T-194; A-127 to T-194; L-128 to T-194; Y-129 to T-194; K-130 to T-194; N-131 to T-194; N-132 to T-194; V-133 to T-194; P-134 to T-194; A-135 to T-194; T-136 to T-194; Y-137 to T-194; T-138 to T-194; Y-139 to T-194; D-140 to T-194; E-141 to T-194; Y-142 to T-194; K-143 to T-194; K-144 to T-194; G-145 to T-194; Y-146 to T-194; L-147 to T-194; D-148 to T-194; Q-149 to T-194; A-150 to T-194; S-151 to T-194; G-152 to T-194; G-153 to T-194; A-154 to T-194; V-155 to T-194; L-156 to T-194; Q-157 to T-194; L-158 to T-194; R-159 to T-194; P-160 to T-194; N-161 to T-194; D-162 to T-194; Q-163 to T-194; V-164 to T-194; W-165 to T-194; V-166 to T-194; Q-167 to T-194; M-168 to T-194; P-169 to T-194; S-170 to T-194; D-171 to T-194; Q-172 to T-194; A-173 to T-194; N-174 to T-194; G-175 to T-194; L-176 to T-194; Y-177 to T-194; S-178 to T-194; T-179 to T-194; E-180 to T-194; Y-181 to T-194; I-182 to T-194; H-183 to T-194; S-184 to T-194; S-185 to T-194; F-186 to T-194; S-187 to T-194; G-188 to T-194; and F-189 to T-194 of SEQ ID NO: 66.

[0389] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0390] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 66: F-55 to P-193; F-55 to C-192; F-55 to L-191; F-55 to L-190; F-55 to F-189; F-55 to G-188; F-55 to S-187; F-55 to F-186; F-55 to S-185; F-55 to S-184; F-55 to H-183; F-55 to I-182; F-55 to Y-181; F-55 to E-180; F-55 to T-179; F-55 to S-178; F-55 to Y-177; F-55 to L-176; F-55 to G-175; F-55 to N-174; F-55 to A-173; F-55 to Q-172; F-55 to D-171; F-55 to S-170; F-55 to P-169; F-55 to M-168; F-55 to Q-167; F-55 to V-166; F-55 to W-165; F-55 to V-164; F-55 to Q-163; F-55 to D-162; F-55 to N-161; F-55 to P-160; F-55 to R-159; F-55 to L-158; F-55 to Q-157; F-55 to L-156; F-55 to V-155; F-55 to A-154; F-55 to G-153; F-55 to G-152; F-55 to S-151; F-55 to A-150; F-55 to Q-149; F-55 to D-148; F-55 to L-147; F-55 to Y-146; F-55 to G-145; F-55 to K-144; F-55 to K-143; F-55 to Y-142; F-55 to E-141; F-55 to D-140; F-55 to Y-139; F-55 to T-138; F-55 to Y-137; F-55 to T-136; F-55 to A-135; F-55 to P-134; F-55 to V-133; F-55 to N-132; F-55 to N-131; F-55 to K-130; F-55 to Y-129; F-55 to L-128; F-55 to A-127; F-55 to V-126; F-55 to W-125; F-55 to V-124; F-55 to N-123; F-55 to T-122; F-55 to G-121; F-55 to K-120; F-55 to V-119; F-55 to H-118; F-55 to V-117; F-55 to H-116; F-55 to Y-115; F-55 to A-114; F-55 to F-113; F-55 to Y-112; F-55 to Y-111; F-55 to V-110; F-55 to G-109; F-55 to G-108; F-55 to V-107; F-55 to P-106; F-55 to C-105; F-55 to T-104; F-55 to F-103; F-55 to I-102; F-55 to G-101; F-55 to T-100; F-55 to A-99; F-55 to P-98; F-55 to N-97; F-55 to Y-96; F-55 to G-95; F-55 to S-94; F-55 to H-93; F-55 to G-92; F-55 to N-91; F-55 to Y-90; F-55 to L-89; F-55 to T-88; F-55 to R-87; F-55 to D-86; F-55 to F-85; F-55 to K-84; F-55 to V-83;

F-55 to P-82; F-55 to M-81; F-55 to G-80; F-55 to S-79; F-55 to A-78; F-55 to P-77; F-55 to F-76; F-55 to P-75; F-55 to S-74; F-55 to T-73; F-55 to L-72; F-55 to V-71; F-55 to A-70; F-55 to T-69; F-55 to F-68; F-55 to A-67; F-55 to P-66; F-55 to T-65; F-55 to A-64; F-55 to H-63; F-55 to A-62; and F-55 to S-61 of SEQ ID NO: 66.

[0391] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0392] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 66, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0393] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a

polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0394] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0395] It has been discovered that this gene is expressed in adipocytes, macrophages, osteoblasts, chondrosarcoma, trabecular bone cells, and cerebellum.

[0396] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, as well as immune, bone, and neural disorders.

[0397] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, immune, musculoskeletal, and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, muscle, bone, neural, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0398] The expression of this gene in adipocytes and the structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well

as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0399] In addition, expression of this gene in macrophages and similarity to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma,

psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0400] Furthermore, the expression of this gene in a number of bone-related tissues and homology to alpha 2 (VIII) collagen indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of bone and joint disorders, including osteoporosis, arthritis, and cancers of bone tissue, such as described under "Hyperproliferative Disorders" herein.

[0401] Alternatively, the expression of this gene in cerebellum, and homology to alpha 2 (VIII) collagen indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of neurodegenerative disorders, such as for example, those described herein under "Neural Activity and Neurological Diseases".

[0402] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 18

[0403] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary

connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0404] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0405] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 67 as residues: Gly-15 to Gln-23, Pro-47 to Gly-69, Gln-98 to Glu-106, Phe-136 to Phe-150, Tyr-186 to Asp-195, Gly-217 to Gly-223, and Ala-226 to Ser-232. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the

invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0406] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 67 as residues Lys-101 to Asn-244. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0407] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 67 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0408] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0409] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g.,

biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0410] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 67: G-102 to N-244; E-103 to N-244; P-104 to N-244; G-105 to N-244; E-106 to N-244; G-107 to N-244; A-108 to N-244; Y-109 to N-244; V-110 to N-244; Y-111 to N-244; R-112 to N-244; S-113 to N-244; A-114 to N-244; F-115 to N-244; S-116 to N-244; V-117 to N-244; G-118 to N-244; L-119 to N-244; E-120 to N-244; T-121 to N-244; Y-122 to N-244; V-123 to N-244; T-124 to N-244; I-125 to N-244; P-126 to N-244; N-127 to N-244; M-128 to N-244; P-129 to N-244; I-130 to N-244; R-131 to N-244; F-132 to N-244; T-133 to N-244; K-134 to N-244; I-135 to N-244; F-136 to N-244; Y-137 to N-244; N-138 to N-244; Q-139 to N-244; Q-140 to N-244; N-141 to N-244; H-142 to N-244; Y-143 to N-244; D-144 to N-244; G-145 to N-244; S-146 to N-244; T-147 to N-244; G-148 to N-244; K-149 to N-244; F-150 to N-244; H-151 to N-244; C-152 to N-244; N-153 to N-244; I-154 to N-244; P-155 to N-244; G-156 to N-244; L-157 to N-244; Y-158 to N-244; Y-159 to N-244; F-160 to N-244; A-161 to N-244; Y-162 to N-244; H-163 to N-244; I-164 to N-244; T-165 to N-244; V-166 to N-244; Y-167 to N-244; M-168 to N-244; K-169 to N-244; D-170 to N-244; V-171 to N-244; K-172 to N-244; V-173 to N-244; S-174 to N-244; L-175 to N-244; F-176 to N-244; K-177 to N-244; K-178 to N-244; D-179 to N-244; K-180 to N-244; A-181 to N-244; M-182 to N-244; L-183 to N-244; F-184 to N-244; T-185 to N-244; Y-186 to N-244; D-187 to N-244; Q-188 to N-244; Y-189 to N-244; Q-190 to N-244; E-191 to N-244; N-192 to N-244; N-193 to

N-244; V-194 to N-244; D-195 to N-244; Q-196 to N-244; A-197 to N-244; S-198 to N-244; G-199 to N-244; S-200 to N-244; V-201 to N-244; L-202 to N-244; L-203 to N-244; H-204 to N-244; L-205 to N-244; E-206 to N-244; V-207 to N-244; G-208 to N-244; D-209 to N-244; Q-210 to N-244; V-211 to N-244; W-212 to N-244; L-213 to N-244; Q-214 to N-244; V-215 to N-244; Y-216 to N-244; G-217 to N-244; E-218 to N-244; G-219 to N-244; E-220 to N-244; R-221 to N-244; N-222 to N-244; G-223 to N-244; L-224 to N-244; Y-225 to N-244; A-226 to N-244; D-227 to N-244; N-228 to N-244; D-229 to N-244; N-230 to N-244; D-231 to N-244; S-232 to N-244; T-233 to N-244; F-234 to N-244; T-235 to N-244; G-236 to N-244; F-237 to N-244; L-238 to N-244; and L-239 to N-244 of SEQ ID NO: 67.

[0411] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0412] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 67: K-101 to T-243; K-101 to D-242; K-101 to H-241; K-101 to Y-240; K-101 to L-239; K-101 to L-238; K-101 to F-237; K-101 to G-236; K-101 to T-235; K-101 to F-234; K-101 to T-233; K-101 to S-232; K-101 to D-231; K-101 to N-230; K-101 to D-229; K-101 to N-228; K-101 to D-227; K-101 to A-226; K-101 to Y-225; K-101 to L-224; K-101 to G-223; K-101 to N-222; K-101 to R-221; K-101 to E-220; K-101 to G-219; K-101 to E-218; K-101 to G-217; K-101 to Y-216; K-101 to V-215; K-101 to Q-214; K-101 to L-213; K-101 to W-212; K-101 to V-211; K-101 to Q-210; K-101 to D-209; K-101 to G-208; K-101 to V-207; K-101 to E-206; K-101 to L-205; K-101 to H-204; K-101 to L-203; K-101 to L-202; K-101 to V-201; K-101 to S-200; K-101 to G-199; K-101 to S-

198; K-101 to A-197; K-101 to Q-196; K-101 to D-195; K-101 to V-194; K-101 to N-193; K-101 to N-192; K-101 to E-191; K-101 to Q-190; K-101 to Y-189; K-101 to Q-188; K-101 to D-187; K-101 to Y-186; K-101 to T-185; K-101 to F-184; K-101 to L-183; K-101 to M-182; K-101 to A-181; K-101 to K-180; K-101 to D-179; K-101 to K-178; K-101 to K-177; K-101 to F-176; K-101 to L-175; K-101 to S-174; K-101 to V-173; K-101 to K-172; K-101 to V-171; K-101 to D-170; K-101 to K-169; K-101 to M-168; K-101 to Y-167; K-101 to V-166; K-101 to T-165; K-101 to I-164; K-101 to H-163; K-101 to Y-162; K-101 to A-161; K-101 to F-160; K-101 to Y-159; K-101 to Y-158; K-101 to L-157; K-101 to G-156; K-101 to P-155; K-101 to I-154; K-101 to N-153; K-101 to C-152; K-101 to H-151; K-101 to F-150; K-101 to K-149; K-101 to G-148; K-101 to T-147; K-101 to S-146; K-101 to G-145; K-101 to D-144; K-101 to Y-143; K-101 to H-142; K-101 to N-141; K-101 to Q-140; K-101 to Q-139; K-101 to N-138; K-101 to Y-137; K-101 to F-136; K-101 to I-135; K-101 to K-134; K-101 to T-133; K-101 to F-132; K-101 to R-131; K-101 to I-130; K-101 to P-129; K-101 to M-128; K-101 to N-127; K-101 to P-126; K-101 to I-125; K-101 to T-124; K-101 to V-123; K-101 to Y-122; K-101 to T-121; K-101 to E-120; K-101 to L-119; K-101 to G-118; K-101 to V-117; K-101 to S-116; K-101 to F-115; K-101 to A-114; K-101 to S-113; K-101 to R-112; K-101 to Y-111; K-101 to V-110; K-101 to Y-109; K-101 to A-108; and K-101 to G-107 of SEQ ID NO: 67.

[0413] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0414] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-

terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 67, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0415] The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0416] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0417] It has been discovered that this gene is expressed in adipose tissue.

[0418] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism.

[0419] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0420] The expression of this gene in adipose tissue and the structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and

skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0421] In addition, expression of this gene in macrophages and similarity to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0422] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 19

[0423] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including EMILIN (e.g., see Genbank Accession No. AF088916), a secreted protein that regulates the formation of elastic fibers in various tissues including blood vessels, cartilage, skin, and lung; and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family.

Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0424] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0425] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 68 as residues: Asn-35 to Arg-53, Arg-108 to Gln-115, Pro-170 to Glu-191, Pro-225 to Gly-230, Asp-241 to Glu-248, Asp-289 to Asn-300, and Pro-302 to Pro-310. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and

variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0426] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 68 as residues Glu-206 to Ala-361. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0427] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 68 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0428] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity

(ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0429] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0430] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 68: G-207 to A-361; A-208 to A-361; P-209 to A-361; A-210 to A-361; A-211 to A-361; P-212 to A-361; V-213 to A-361; P-214 to A-361; Q-215 to A-361; V-216 to A-361; A-217 to A-361; F-218 to A-361; S-219 to A-361; A-220 to A-361; A-221 to A-361; L-222 to A-361; S-223 to A-361; L-224 to A-361; P-225 to A-361; R-226 to A-361; S-227 to A-361; E-228 to A-361; P-229 to A-361; G-230 to A-361; T-231 to A-361; V-232 to A-361; P-233 to A-361; F-234 to A-361; D-235 to A-361; R-236 to A-361; V-237 to A-361; L-238 to A-361; L-239 to A-361; N-240 to A-361; D-241 to A-361; G-242 to A-361; G-243 to A-361; Y-244 to A-361; Y-245 to A-361; D-246 to A-361; P-247 to A-361; E-248 to A-361; T-249 to A-361; G-250 to A-361; V-251 to A-361; F-252 to A-361; T-253 to A-361; A-254 to A-361; P-255 to A-361; L-256 to A-361; A-257 to A-361; G-258 to A-361; R-259 to A-361; Y-260 to A-361; L-261 to A-361; L-262 to A-361; S-263 to A-361; A-264 to A-361; V-265 to A-361; L-266 to A-361; T-267 to A-361; G-268 to A-361; H-269 to A-361; R-270 to A-361; H-271 to A-361; E-272 to

A-361; K-273 to A-361; V-274 to A-361; E-275 to A-361; A-276 to A-361; V-277 to A-361; L-278 to A-361; S-279 to A-361; R-280 to A-361; S-281 to A-361; N-282 to A-361; Q-283 to A-361; G-284 to A-361; V-285 to A-361; A-286 to A-361; R-287 to A-361; V-288 to A-361; D-289 to A-361; S-290 to A-361; G-291 to A-361; G-292 to A-361; Y-293 to A-361; E-294 to A-361; P-295 to A-361; E-296 to A-361; G-297 to A-361; L-298 to A-361; E-299 to A-361; N-300 to A-361; K-301 to A-361; P-302 to A-361; V-303 to A-361; A-304 to A-361; E-305 to A-361; S-306 to A-361; Q-307 to A-361; P-308 to A-361; S-309 to A-361; P-310 to A-361; G-311 to A-361; T-312 to A-361; L-313 to A-361; G-314 to A-361; V-315 to A-361; F-316 to A-361; S-317 to A-361; L-318 to A-361; I-319 to A-361; L-320 to A-361; P-321 to A-361; L-322 to A-361; Q-323 to A-361; A-324 to A-361; G-325 to A-361; D-326 to A-361; T-327 to A-361; V-328 to A-361; C-329 to A-361; V-330 to A-361; D-331 to A-361; L-332 to A-361; V-333 to A-361; M-334 to A-361; G-335 to A-361; Q-336 to A-361; L-337 to A-361; A-338 to A-361; H-339 to A-361; S-340 to A-361; E-341 to A-361; E-342 to A-361; P-343 to A-361; L-344 to A-361; T-345 to A-361; I-346 to A-361; F-347 to A-361; S-348 to A-361; G-349 to A-361; A-350 to A-361; L-351 to A-361; L-352 to A-361; Y-353 to A-361; G-354 to A-361; D-355 to A-361; and P-356 to A-361 of SEQ ID NO: 68.

[0431] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0432] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 68: E-206 to H-360; E-206 to E-359; E-206 to L-358; E-206 to E-357; E-206 to P-356; E-206 to D-355; E-206 to G-354; E-206 to Y-353; E-206 to L-352; E-206 to

L-351; E-206 to A-350; E-206 to G-349; E-206 to S-348; E-206 to F-347; E-206 to I-346; E-206 to T-345; E-206 to L-344; E-206 to P-343; E-206 to E-342; E-206 to E-341; E-206 to S-340; E-206 to H-339; E-206 to A-338; E-206 to L-337; E-206 to Q-336; E-206 to G-335; E-206 to M-334; E-206 to V-333; E-206 to L-332; E-206 to D-331; E-206 to V-330; E-206 to C-329; E-206 to V-328; E-206 to T-327; E-206 to D-326; E-206 to G-325; E-206 to A-324; E-206 to Q-323; E-206 to L-322; E-206 to P-321; E-206 to L-320; E-206 to I-319; E-206 to L-318; E-206 to S-317; E-206 to F-316; E-206 to V-315; E-206 to G-314; E-206 to L-313; E-206 to T-312; E-206 to G-311; E-206 to P-310; E-206 to S-309; E-206 to P-308; E-206 to Q-307; E-206 to S-306; E-206 to E-305; E-206 to A-304; E-206 to V-303; E-206 to P-302; E-206 to K-301; E-206 to N-300; E-206 to E-299; E-206 to L-298; E-206 to G-297; E-206 to E-296; E-206 to P-295; E-206 to E-294; E-206 to Y-293; E-206 to G-292; E-206 to G-291; E-206 to S-290; E-206 to D-289; E-206 to V-288; E-206 to R-287; E-206 to A-286; E-206 to V-285; E-206 to G-284; E-206 to Q-283; E-206 to N-282; E-206 to S-281; E-206 to R-280; E-206 to S-279; E-206 to L-278; E-206 to V-277; E-206 to A-276; E-206 to E-275; E-206 to V-274; E-206 to K-273; E-206 to E-272; E-206 to H-271; E-206 to R-270; E-206 to H-269; E-206 to G-268; E-206 to T-267; E-206 to L-266; E-206 to V-265; E-206 to A-264; E-206 to S-263; E-206 to L-262; E-206 to L-261; E-206 to Y-260; E-206 to R-259; E-206 to G-258; E-206 to A-257; E-206 to L-256; E-206 to P-255; E-206 to A-254; E-206 to T-253; E-206 to F-252; E-206 to V-251; E-206 to G-250; E-206 to T-249; E-206 to E-248; E-206 to P-247; E-206 to D-246; E-206 to Y-245; E-206 to Y-244; E-206 to G-243; E-206 to G-242; E-206 to D-241; E-206 to N-240; E-206 to L-239; E-206 to L-238; E-206 to V-237; E-206 to R-236; E-206 to D-235; E-206 to F-234; E-206 to P-233; E-206 to V-232; E-206 to T-231; E-206 to G-230; E-206 to P-229; E-206 to E-228; E-206 to S-227; E-206 to R-226; E-206 to P-225; E-206 to L-224; E-206 to S-223; E-206 to L-222; E-206 to A-221; E-206 to A-220; E-206 to S-219; E-206 to F-218; E-206 to A-217; E-206 to V-216; E-206 to Q-215; E-206 to P-214; E-206 to V-213; and E-206 to P-212 of SEQ ID NO: 68.

[0433] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under

stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0434] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 68, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0435] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-791, where this portion excludes any integer of amino acid residues from 1 to about 355 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-791, or any integer of amino acid residues from 1 to about 355 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-791. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0436] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0437] It has been discovered that this gene is expressed in adipose tissue.

[0438] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism.

[0439] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0440] The expression of this gene in adipose tissue and the structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to,

seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0441] In addition, the similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0442] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO.: 20

[0443] The translation product of this gene shares sequence and/or structural similarity with members of the C1q family of proteins, including collagen alpha 1(X) (e.g., see Genbank Accession No. S23297), a matrix protein involved in tissue remodeling such as

in injured arteries and atherosclerotic plaques; and ACRP30, an adipocyte complement-related protein (see, e.g., Genbank Accession Numbers AAA80543 and Q15848; all references available through these accessions are herein incorporated by reference in their entireties). ACRP30 is an abundant serum protein secreted exclusively from fat cells, which is implicated in energy homeotasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody-antigen complexes in the classical pathway of complement. The structure reveals a homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. It has been suggested that TNFs, which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis arose by divergence from a primordial recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins (Shapiro and Scherer, Curr Biol 8:335-338 (1998).

[0444] The ACRP30 cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a stalk region (Gly-X-Y repeats), and a globular domain. The globular domain of ACRP30 is situated at the COOH-terminus and shares significant homology with subunits of complement factor C1q, collagen 1(X), the brain-specific factor cerebellin, hibernating proteins-20, 25, and 27, TNF alpha, CORS26, collagen VIII, Elastin Microfibril Interface-Located Proteins (EMILINs), and Multimerin. The expression of ACRP30 is highly specific to adipose tissue in both mouse and rat. Expression of ACRP30 is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain ACRP30 mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for ACRP30. Furthermore, the expression of ACRP30 mRNA is significantly reduced in the adipose tissues from obese mice and humans. The tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue (Liang and Spiegelman, J. Biol Chem. 271:10697-10703 (1996)). ACRP-30 is believed to play a role in clearing lipids from the blood by increasing free fatty acid (FFA) oxidation by muscle tissue. Also, FFAs are potent inhibitors of insulin signaling. Accordingly, homologs of ACRP-30, and corresponding

agonists thereof such as antibodies, are useful for treating obesity and diabetes, as well as other metabolic and endocrine conditions or disorders.

[0445] Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, or more of the immunogenic epitopes shown in SEQ ID NO: 69 as residues: Asn-16 to Gly-26, Asn-33 to Gly-38, Pro-40 to Gly-80, Gly-89 to Lys-103, Glu-117 to Val-130, Pro-132 to Asn-141, Pro-160 to Glu-165, Pro-168 to Gly-173, Gly-182 to Lys-192, Glu-230 to Asp-235, Met-281 to Ala-287, and Asp-317 to Thr-322. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0446] In nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, the globular domain of the protein shown in SEQ ID NO: 69 as residues Lys-190 to Pro-333. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0447] Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature portion of the protein shown in SEQ ID NO: 69 demonstrating functional activity. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention.

Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0448] By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity (e.g., modulating glucose transport in adipocytes, clearing lipids from the blood, ability to increase FFA oxidation by muscle tissue, and increased expression in response to insulin), antigenicity (ability to bind, or compete with a polypeptide of the invention for binding, to an antibody specific for the polypeptide of the invention), immunogenicity (ability to generate antibodies which specifically bind to the polypeptides of the invention), and ability to form multimers with polypeptides of the invention.

[0449] Even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to increase FFA oxidation by muscle tissue), other functional activities (e.g., biological activities, ability to multimerize, ability to induce antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0450] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 69: G-191 to P-333; K-192 to P-333; I-193 to P-333; G-194 to P-333; E-195 to P-333; T-196 to P-333; L-197 to P-333; V-198 to P-333; L-199 to P-333; P-200 to P-333; K-201 to P-333; S-202 to P-333; A-203 to P-333; F-204 to P-333; T-205 to P-333; V-206 to P-333; G-207 to P-333; L-208 to P-333; T-209 to P-333; V-210

to P-333; L-211 to P-333; S-212 to P-333; K-213 to P-333; F-214 to P-333; P-215 to P-333; S-216 to P-333; S-217 to P-333; D-218 to P-333; V-219 to P-333; P-220 to P-333; I-221 to P-333; K-222 to P-333; F-223 to P-333; D-224 to P-333; K-225 to P-333; I-226 to P-333; L-227 to P-333; Y-228 to P-333; N-229 to P-333; E-230 to P-333; F-231 to P-333; N-232 to P-333; H-233 to P-333; Y-234 to P-333; D-235 to P-333; T-236 to P-333; A-237 to P-333; A-238 to P-333; G-239 to P-333; K-240 to P-333; F-241 to P-333; T-242 to P-333; C-243 to P-333; H-244 to P-333; I-245 to P-333; A-246 to P-333; G-247 to P-333; V-248 to P-333; Y-249 to P-333; Y-250 to P-333; F-251 to P-333; T-252 to P-333; Y-253 to P-333; H-254 to P-333; I-255 to P-333; T-256 to P-333; V-257 to P-333; F-258 to P-333; S-259 to P-333; R-260 to P-333; N-261 to P-333; V-262 to P-333; Q-263 to P-333; V-264 to P-333; S-265 to P-333; L-266 to P-333; V-267 to P-333; K-268 to P-333; N-269 to P-333; G-270 to P-333; V-271 to P-333; K-272 to P-333; I-273 to P-333; L-274 to P-333; H-275 to P-333; T-276 to P-333; K-277 to P-333; D-278 to P-333; A-279 to P-333; Y-280 to P-333; M-281 to P-333; S-282 to P-333; S-283 to P-333; E-284 to P-333; D-285 to P-333; Q-286 to P-333; A-287 to P-333; S-288 to P-333; G-289 to P-333; G-290 to P-333; I-291 to P-333; V-292 to P-333; L-293 to P-333; Q-294 to P-333; L-295 to P-333; K-296 to P-333; L-297 to P-333; G-298 to P-333; D-299 to P-333; E-300 to P-333; V-301 to P-333; W-302 to P-333; L-303 to P-333; Q-304 to P-333; V-305 to P-333; T-306 to P-333; G-307 to P-333; G-308 to P-333; E-309 to P-333; R-310 to P-333; F-311 to P-333; N-312 to P-333; G-313 to P-333; L-314 to P-333; F-315 to P-333; A-316 to P-333; D-317 to P-333; E-318 to P-333; D-319 to P-333; D-320 to P-333; D-321 to P-333; T-322 to P-333; T-323 to P-333; F-324 to P-333; T-325 to P-333; G-326 to P-333; F-327 to P-333; and L-328 to P-333 of SEQ ID NO: 69.

[0451] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention.

Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0452] Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of C-terminal deletions of the globular domain of the polypeptide of the invention shown as SEQ ID NO: 69: K-190 to S-332; K-190 to S-331; K-190 to F-330; K-190 to L-329; K-190 to L-328; K-190 to F-327; K-190 to G-326; K-190 to T-325; K-190 to F-324; K-190 to T-323; K-190 to T-322; K-190 to D-321; K-190 to D-320; K-190 to D-319; K-190 to E-318; K-190 to D-317; K-190 to A-316; K-190 to F-315; K-190 to L-314; K-190 to G-313; K-190 to N-312; K-190 to F-311; K-190 to R-310; K-190 to E-309; K-190 to G-308; K-190 to G-307; K-190 to T-306; K-190 to V-305; K-190 to Q-304; K-190 to L-303; K-190 to W-302; K-190 to V-301; K-190 to E-300; K-190 to D-299; K-190 to G-298; K-190 to L-297; K-190 to K-296; K-190 to L-295; K-190 to Q-294; K-190 to L-293; K-190 to V-292; K-190 to I-291; K-190 to G-290; K-190 to G-289; K-190 to S-288; K-190 to A-287; K-190 to Q-286; K-190 to D-285; K-190 to E-284; K-190 to S-283; K-190 to S-282; K-190 to M-281; K-190 to Y-280; K-190 to A-279; K-190 to D-278; K-190 to K-277; K-190 to T-276; K-190 to H-275; K-190 to L-274; K-190 to I-273; K-190 to K-272; K-190 to V-271; K-190 to G-270; K-190 to N-269; K-190 to K-268; K-190 to V-267; K-190 to L-266; K-190 to S-265; K-190 to V-264; K-190 to Q-263; K-190 to V-262; K-190 to N-261; K-190 to R-260; K-190 to S-259; K-190 to F-258; K-190 to V-257; K-190 to T-256; K-190 to I-255; K-190 to H-254; K-190 to Y-253; K-190 to T-252; K-190 to F-251; K-190 to Y-250; K-190 to Y-249; K-190 to V-248; K-190 to G-247; K-190 to A-246; K-190 to I-245; K-190 to H-244; K-190 to C-243; K-190 to T-242; K-190 to F-241; K-190 to K-240; K-190 to G-239; K-190 to A-238; K-190 to A-237; K-190 to T-236; K-190 to D-235; K-190 to Y-234; K-190 to H-233; K-190 to N-232; K-190 to F-231; K-190 to E-230; K-190 to N-229; K-190 to Y-228; K-190 to L-227; K-190 to I-226; K-190 to K-225; K-190 to D-224; K-190 to F-223; K-190 to K-222; K-190 to I-221; K-190 to P-220; K-190 to V-219; K-190 to D-218; K-190 to S-217; K-190 to S-216; K-190 to P-215; K-190 to F-214; K-190 to K-213; K-190 to S-212; K-190 to L-211; K-190 to V-210; K-190 to T-209; K-190 to L-208; K-190 to G-207; K-190 to V-206; K-190 to T-205; K-190 to F-204; K-190 to A-203; K-190 to S-202; K-190 to K-201; K-190 to P-200; K-190 to L-199; K-190 to V-198; K-190 to L-197; and K-190 to T-196 of SEQ ID NO: 69.

[0453] Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (e.g., fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof) are encompassed by the invention. Antibodies that bind these fragments and variants of the invention are also encompassed by the invention. Polynucleotides encoding these fragments and variants are also encompassed by the invention.

[0454] N-terminal deletions of translation products of the instant invention may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. C-terminal deletions of translation products of the instant invention may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 69, where n and m are integers as described above. Fragments and/or variants of these polypeptides, such as, for example, fragments and/or variants as described herein, are encompassed by the invention. Polynucleotides encoding these polypeptides (including fragments and/or variants) are also encompassed by the invention, as are antibodies that bind these polypeptides.

[0455] Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3449, where this portion excludes any integer of amino acid residues from 1 to about 327 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. PTA-3449, or any integer of amino acid residues from 1 to about 327 amino acids from the carboxy

terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. PTA-3449. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

[0456] As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

[0457] It has been discovered that this gene is expressed in adipose tissue, bone marrow stem cells, neutrophils, skeletal muscle, and adult heart.

[0458] Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: diabetes, obesity, and diseases and/or disorders involving dysfunctional fatty acid metabolism, as well as immunological and cardiovascular disorders.

[0459] Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine, immune, and cardiovascular systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., adipose, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

[0460] The expression of this gene in adipose tissue and muscle tissue and the structural similarity of translation products of this gene to ACRP-30 indicates that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of diabetes and diabetes-related disorders, as well as obesity and other metabolic disorders, such as, for example, those described herein under "Endocrine Disorders". Polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, and/or ameliorate both type I Insulin-Dependent Diabetes Mellitus, "IDDM", and type II Non-Insulin-Dependent Diabetes

Mellitus, "NIDDM". Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene, as well as agonists or antagonists thereof (including antibodies and small molecule drugs) may be used to treat, prevent, or ameliorate conditions associated with either type I Insulin-Dependent Diabetes Mellitus, "IDDM", or type II Non-Insulin-Dependent Diabetes Mellitus, "NIDDM", including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations. In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0461] In addition, the expression of this gene in bone marrow stem cells and neutrophils, and similarity of this gene to other members of the C1q family of proteins suggests that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis, prevention, and/or treatment of immunological disorders, including inflammation, infection, autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis, and/or those described herein under "Immune Activity".

[0462] Alternatively, the expression of this gene in heart and muscle tissue, and homology to alpha 1 (X) collagen indicate that polynucleotides and polypeptides corresponding to this gene, including antibodies, are useful for the diagnosis, prognosis,

prevention, and/or treatment of cardiovacular disorders, such as for example, atherosclerosis, restenosis, and/or those disclosed herein under "Cardiovascular Disorders".

[0463] Translation products of this gene, as well as antibodies directed against translation products of this gene, may show utility as tumor markers and/or immunotherapy targets for the above listed tissues.

TABLE 1

Gene No.	cDNA Plasmid: V	ATCC Deposit No. and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HCE1P80	PTA-844 10/13/99	Uni-ZAP XR	2	3522	1	3522	93	93	50	1	19	20	229
1	HCE1P80	PTA-844 10/13/99	Uni-ZAP XR	22	3543	1	3543	108	108	70	1	21	22	229
1	HCE1P80	PTA-844 10/13/99	Uni-ZAP XR	23	3522	1	3522	93	93	71	1	21	22	229
2	HUFGH53	PTA-623 9/2/99	pSport1	3	2007	1	1968	36	36	51	1	22	23	421
2	HUFGH53	PTA-623 9/2/99	pSport1	24	1969	1	1969	36	36	72	1	22	23	459
2	HUFGH53	PTA-623 9/2/99	pSport1	25	2189	1	2189	108	108	73	1	22	23	443
2	HUFGH53	PTA-623 9/2/99	pSport1	26	1236	397	1236		108	74	1			12
2	HUFGH53	PTA-623 9/2/99	pSport1	27	832	1	832		257	75	1	16	17	19
2	HUFGH53	PTA-623 9/2/99	pSport1	28	1967	1	1967	36	36	76	1	22	23	421
2	HUFGH53	PTA-623 9/2/99	pSport1	29	1967	1	1967	36	36	77	1	22	23	421

Gene No.	cDNA Plasmid: V	ATCC Deposit No. and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
2	HUFGH53	PTA-623 9/2/99	pSport1	30	2006	1	1967	36	36	78	1	22	23	458
3	HWMMO59		pSport1	4	901	1	901		2	52	1	1	2	240
3	HWMMO59		pSport1	31	897	1	897		2	79	1	1	2	240
4	HSSJJ51	PTA-536 08/13/99	Uni-ZAP XR	5	1558	1	1558	278	278	53	1	25	26	281
5	HCEWD38	PTA-3696 9/10/01	Uni-ZAP XR	6	1313	1	1313	120	120	54	1	32	33	205
5	HCEWD38	PTA-3696 9/10/01	Uni-ZAP XR	32	990	68	990	193	193	80	1	32	33	256
5	HCEWD38	PTA-3696 9/10/01	Uni-ZAP XR	33	1384	68	1179	193	193	81	1	35	36	205
6	HUCMC56	PTA-2574 PTA2575 10/5/00	pSport1	7	1312	1	1312		2	55	1	29	30	189
6	HUCMC56	PTA-2574 PTA2575 10/5/00	pSport1	34	809	1	809		2	82	1	1	2	180
7	HWLZU06	PTA-3696 9/10/01	pSport1	8	1022	1	1022		3	56	1	11	12	201
7	HWLZU06	PTA-3696 9/10/01	pSport1	35	1215	266	1215		82	83	1			241

Gene No.	cDNA Plasmid: V	ATCC Deposit No. and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
8	HDPBA69	PTA-3696 9/10/01	pCMVSport 3.0	9	1254	1	1254	73	73	57	1	28	29	245
8	HDPBA69	PTA-3696 9/10/01	pCMVSport 3.0	36	1311	54	1311	119	119	84	1	28	29	245
8	HDPBA69	PTA-3696 9/10/01	pCMVSport 3.0	37	350	240	350		17	85	1	6	7	76
8	HDPBA69	PTA-3696 9/10/01	pCMVSport 3.0	38	622	277	622	68	68	86	1	28	29	185
9	HLWAE11	203071 07/27/98	pCMVSport 3.0	10	1618	1	1618	28	28	58	1	46	47	278
10	HSZAF47	209124 06/19/97	Uni-ZAP XR	11	1336	1	1336	113	113	59	1	16	17	289
10	HSZAF47	209124 06/19/97	Uni-ZAP XR	39	1333	2	1333	107	107	87	1	18	19	127
11	HWTAY65	PTA-3696 9/10/01	Uni-ZAP XR	12	1114	1	1114	66	66	60	1	17	18	285
11	HWTAY65	PTA-3696 9/10/01	Uni-ZAP XR	40	1211	1	1211	158	158	88	1	17	18	285
12	HHGDP51	PTA-3696 9/10/01	Lambda ZAP II	13	659	1	659		9	61	1	13	14	146
12	HHGDP51	PTA-3696 9/10/01	Lambda ZAP II	41	616	250	616		1	89	1	1	2	205

Gene No.	cDNA Plasmid: V	ATCC Deposit No. and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
13	HBCBS41	PTA-2574 PTA2575 10/5/00	Uni-ZAP XR	14	1195	551	1195	217	217	62	1	25	26	251
13	HBCBS41	PTA-2574 PTA2575 10/5/00	Uni-ZAP XR	42	1161	513	1161	179	179	90	1	25	26	251
13	HBCBS41	PTA-2574 PTA2575 10/5/00	Uni-ZAP XR	43	687	89	687		2	91	1	1	2	168
13	HDMBJ47	PTA-2574 PTA2575 10/5/00	pSport1	44	1194	1	1194		158	92	1	1	2	255
14	HDPRZ06	PTA-2574 PTA2575 10/5/00	pCMVSport 3.0	15	3951	2373	3951	135	135	63	1	30	31	975
14	HDPRZ06	PTA-2574 PTA2575 10/5/00	pCMVSport 3.0	45	1792	1	1792		209	93	1	1	2	258
14	HDPRZ06	PTA-2574 PTA2575 10/5/00	pCMVSport 3.0	46	1412	1	1412		3	94	1	1	2	232
15	HKB1F69	PTA-2574 PTA2575 10/5/00	pCMVSport 1	16	1280	1	1230	386	386	64	1	1	2	158
16	HOHBO69	PTA-2574 PTA2575 10/5/00	pCMVSport 2.0	17	3764	1	716	829	829	65	1	1	2	605

Gene No.	cDNA Plasmid: V	ATCC Deposit No. and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
16	HOHBO69	PTA-2574 PTA2575 10/5/00	pCMVSport 2.0	47	646	1	646	353	353	95	1	27	28	98
17	HCEES60		Uni-ZAP XR	18	2485	1	2485		1	66	1	1	2	194
18	HDALV07		pCMVSport 3.0	19	1550	1	1550	42	42	67	1	17	18	244
19	HEQAH47	PTA-791 9/27/99	pCMVSport 3.0	20	1518	1	1518		3	68	1	1	2	361
19	HEQAH47	PTA-791 9/27/99	pCMVSport 3.0	48	2536	1023	2536	472	472	96	1	1	2	542
20	HATNA88	PTA-3449 6/11/01	pCMVSport 3.0	21	1545	1	1545	342	342	69	1	19	20	333
20	HATNA88	PTA-3449 6/11/01	pCMVSport 3.0	49	1530	1	1530	326	326	97	1	19	20	333

[0464] Table 1 summarizes the information corresponding to each "Gene No:" described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA Plasmid:V" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

[0465] The cDNA Plasmid:V was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in cDNA Plasmid:V.

[0466] "Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No:". The deposited plasmid contains all of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative methionine start codon (if present) is identified as "5' NT of Start Codon." Similarly , the nucleotide position of SEQ ID NO:X of the predicted signal sequence (if present) is identified as "5' NT of First AA of Signal Pep."

[0467] The translated amino acid sequence, beginning with the first translated codon of the polynucleotide sequence, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

[0468] SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X has uses including, but not limited to, in designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in a deposited plasmid. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID

NO:Y have uses that include, but are not limited to generating antibodies, which bind specifically to the secreted proteins encoded by the cDNA clones identified in Table 1.

[0469] Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

[0470] Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X, and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited plasmid can readily be determined by sequencing the deposited plasmid in accordance with known methods.

[0471] The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular plasmid can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

[0472] Also provided in Table 1 is the name of the vector which contains the cDNA plasmid. Each vector is routinely used in the art. The following additional information is provided for convenience.

[0473] Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res. 16:*7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., *Nucleic Acids Res. 17:*9494 (1989)) and pBK (Alting-Mees, M. A. et al., *Strategies 5:*58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene.

Phagemid pBS may be excised from the Lambda Zap and Uni-Zap XR vectors, and phagemid pBK may be excised from the Zap Express vector. Both phagemids may be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene.

[0474] Vectors pSport1, pCMVSport 1.0, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus 15:*59 (1993). Vector lafmid BA (Bento Soares, Columbia University, New York, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res. 16:*9677-9686 (1988) and Mead, D. *et al., Bio/Technology 9:* (1991).

[0475] The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or a deposited plasmid (cDNA plasmid:V). The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include, but are not limited to, preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

[0476] Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or cDNA plasmid:V, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

[0477] The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X and/or cDNA plasmid:V. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X,

and/or a polypeptide encoded by the cDNA in cDNA plasmid:V. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X and/or a polypeptide encoded by the cDNA in cDNA plasmid:V, are also encompassed by the invention. The present invention further encompasses a polynucleotide comprising, or alternatively consisting of the complement of the nucleic acid sequence of SEQ ID NO:X, and/or the complement of the coding strand of the cDNA in cDNA plasmid:V.

[0478] Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would unduly burden the disclosure of this application. Accordingly, preferably excluded from SEQ ID NO:X are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 and the final nucleotide minus 15 of SEQ ID NO:X, b is an integer of 15 to the final nucleotide of SEQ ID NO:X, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:X, and where b is greater than or equal to a + 14.

RACE Protocol For Recovery of Full-Length Genes

[0479] Partial cDNA clones can be made full-length by utilizing the rapid amplification of cDNA ends (RACE) procedure described in Frohman, M.A., et al., Proc. Nat'l. Acad. Sci. USA, 85:8998-9002 (1988). A cDNA clone missing either the 5' or 3' end can be reconstructed to include the absent base pairs extending to the translational start or stop codon, respectively. In some cases, cDNAs are missing the start of translation, therefor. The following briefly describes a modification of this original 5' RACE procedure. Poly A+ or total RNA is reverse transcribed with Superscript II (Gibco/BRL) and an antisense or complementary primer specific to the cDNA sequence. The primer is removed from the reaction with a Microcon Concentrator (Amicon). The first-strand cDNA is then tailed with dATP and terminal deoxynucleotide transferase (Gibco/BRL). Thus, an anchor sequence is produced which is needed for PCR amplification. The second strand is synthesized from the dA-tail in PCR buffer, Taq DNA polymerase (Perkin-Elmer Cetus), an oligo-dT primer containing three adjacent restriction sites (XhoI, SalI and ClaI) at the 5'

end and a primer containing just these restriction sites. This double-stranded cDNA is PCR amplified for 40 cycles with the same primers as well as a nested cDNA-specific antisense primer. The PCR products are size-separated on an ethidium bromide-agarose gel and the region of gel containing cDNA products the predicted size of missing protein-coding DNA is removed. cDNA is purified from the agarose with the Magic PCR Prep kit (Promega), restriction digested with XhoI or SalI, and ligated to a plasmid such as pBluescript SKII (Stratagene) at XhoI and EcoRV sites. This DNA is transformed into bacteria and the plasmid clones sequenced to identify the correct protein-coding inserts. Correct 5' ends are confirmed by comparing this sequence with the putatively identified homologue and overlap with the partial cDNA clone. Similar methods known in the art and/or commercial kits are used to amplify and recover 3' ends.

[0480] Several quality-controlled kits are commercially available for purchase. Similar reagents and methods to those above are supplied in kit form from Gibco/BRL for both 5' and 3' RACE for recovery of full length genes. A second kit is available from Clontech which is a modification of a related technique, SLIC (single-stranded ligation to single-stranded cDNA), developed by Dumas et al., Nucleic Acids Res., 19:5227-32 (1991). The major differences in procedure are that the RNA is alkaline hydrolyzed after reverse transcription and RNA ligase is used to join a restriction site-containing anchor primer to the first-strand cDNA. This obviates the necessity for the dA-tailing reaction which results in a polyT stretch that is difficult to sequence past.

[0481] An alternative to generating 5' or 3' cDNA from RNA is to use cDNA library double-stranded DNA. An asymmetric PCR-amplified antisense cDNA strand is synthesized with an antisense cDNA-specific primer and a plasmid-anchored primer. These primers are removed and a symmetric PCR reaction is performed with a nested cDNA-specific antisense primer and the plasmid-anchored primer.

RNA Ligase Protocol For Generating The 5' or 3' End Sequences To Obtain Full Length Genes

[0482] Once a gene of interest is identified, several methods are available for the identification of the 5' or 3' portions of the gene which may not be present in the original cDNA plasmid. These methods include, but are not limited to, filter probing, clone enrichment using specific probes and protocols similar and identical to 5' and 3'RACE.

While the full length gene may be present in the library and can be identified by probing, a useful method for generating the 5' or 3' end is to use the existing sequence information from the original cDNA to generate the missing information. A method similar to 5'RACE is available for generating the missing 5' end of a desired full-length gene. (This method was published by Fromont-Racine et al., Nucleic Acids Res., 21(7):1683-1684 (1993)). Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcript and a primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest, is used to PCR amplify the 5' portion of the desired full length gene which may then be sequenced and used to generate the full length gene. This method starts with total RNA isolated from the desired source, poly A RNA may be used but is not a prerequisite for this procedure. The RNA preparation may then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase if used is then inactivated and the RNA is treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase. This modified RNA preparation can then be used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction can then be used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the ACRP30-Like gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the relevant ACRP30-Like gene.

Polynucleotide and Polypeptide Fragments

[0483] The present invention is also directed to polynucleotide fragments of the polynucleotides (nucleic acids) of the invention. In the present invention, a "polynucleotide fragment" refers to a polynucleotide having a nucleic acid sequence which: is a portion of the cDNA contained in cDNA plasmid:V or encoding the polypeptide encoded by the cDNA contained in cDNA plasmid:V; is a portion of the polynucleotide sequence in SEQ ID NO:X or the complementary strand thereto; is a

polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; or is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, at least about 100 nt, at least about 125 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from, for example, the sequence contained in the cDNA in cDNA plasmid:V, or the nucleotide sequence shown in SEQ ID NO:X or the complementary stand thereto. In this context "about" includes the particularly recited value, or a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 150, 175, 200, 250, 500, 600, 1000, or 2000 nucleotides in length) are also encompassed by the invention.

[0484] Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, 3551-3600, 3601-3650, 3651-3700, 3701-3750, and/or 3751-3764 of SEQ ID NO:X, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g. biological activity) of the polypeptide encoded by a polynucleotide of which the sequence is a portion. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which

hybridize to one or more of these fragments under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

[0485] Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, 3551-3600, 3601-3650, 3651-3700, 3701-3750, and/or 3751-3764 of the cDNA nucleotide sequence contained in cDNA plasmid:V, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g. biological activity) of the polypeptide encoded by the cDNA nucleotide sequence contained in cDNA plasmid:V. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these fragments under stringent hybridization conditions, or alternatively, under lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

[0486] In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y, a portion of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO:X, and/or encoded by the cDNA in cDNA plasmid:V. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively

consisting of, an amino acid sequence from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 101-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, and/or 601-605 of the coding region of SEQ ID NO:Y. Moreover, polypeptide fragments of the invention may be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, or ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either terminus or at both termini. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

[0487] Even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0488] Accordingly, polypeptide fragments of the invention include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

[0489] The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in cDNA plasmid:V). In particular, N-terminal deletions may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

[0490] Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example the ability of the shortened mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0491] Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in cDNA plasmid:V). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

[0492] In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to, the preferred polypeptide disclosed as SEQ ID NO:Y), and/or the cDNA in cDNA plasmid:V, and/or the complement thereof, where n and m are integers as described above. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

[0493] Any polypeptide sequence contained in the polypeptide of SEQ ID NO:Y, encoded by the polynucleotide sequences set forth as SEQ ID NO:X, or encoded by the cDNA in cDNA plasmid:V may be analyzed to determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of SEQ ID NO:X or the cDNA in cDNA plasmid:V may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; http://www.dnastar.com/).

[0494] Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides of the invention in this regard are those that encode polypeptides comprising regions that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

[0495] Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values which represent regions of the polypeptide which

are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

[0496] Preferred polypeptide fragments of the invention are fragments comprising, or alternatively, consisting of, an amino acid sequence that displays a functional activity (e.g. biological activity) of the polypeptide sequence of which the amino acid sequence is a fragment. By a polypeptide displaying a "functional activity" is meant a polypeptide capable of one or more known functional activities associated with a full-length protein, such as, for example, biological activity, antigenicity, immunogenicity, and/or multimerization, as described supra.

[0497] Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

[0498] In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

[0499] The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide sequence shown in SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by the cDNA in cDNA plasmid:V, or encoded by a polynucleotide that hybridizes to the complement of an epitope encoding sequence of SEQ ID NO:X, or an epitope encoding sequence contained in cDNA plasmid:V under stringent hybridization conditions, or alternatively, under lower stringency hybridization, as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to this complementary strand under stringent hybridization conditions, or alternatively, under lower stringency hybridization conditions, as defined supra.

[0500] The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

[0501] Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

[0502] In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

[0503] Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra;

Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

[0504] Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

[0505] As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention and immunogenic and/or antigenic epitope

fragments thereof can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

[0506] Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995)).

[0507] Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides

for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984)).

[0508] Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

[0509] Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., Proc. Natl. Acad. Sci. USA 88:8972- 897 (1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

[0510] Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide

insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Polynucleotide and Polypeptide Variants

[0511] The invention also encompasses ACRP30-Like variants. The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X or the complementary strand thereto, and/or the cDNA sequence contained in cDNA plasmid:V.

[0512] The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y, a polypeptide sequence encoded by the polynucleotide sequence in SEQ ID NO:X and/or a polypeptide sequence encoded by the cDNA in cDNA plasmid:V.

[0513] "Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

[0514] Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence described in SEQ ID NO:X or contained in the cDNA sequence of Plasmid:V; (b) a nucleotide sequence in SEQ ID NO:X or the cDNA in Plasmid:V which encodes the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Plasmid:V; (c) a nucleotide sequence in SEQ ID NO:X or the cDNA in Plasmid:V which encodes a mature ACRP30-Like polypeptide; (d) a nucleotide sequence in SEQ ID NO:X or the cDNA sequence of Plasmid:V, which encodes a biologically active fragment of a ACRP30-Like polypeptide; (e) a nucleotide sequence in SEQ ID NO:X or the cDNA sequence of Plasmid:V, which encodes an antigenic fragment of a ACRP30-Like polypeptide; (f) a nucleotide sequence encoding a ACRP30-Like polypeptide comprising the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Plasmid:V; (g) a nucleotide sequence encoding a mature

ACRP30-Like polypeptide of the amino acid sequence of SEQ ID NO:Y or the amino acid sequence encoded by the cDNA in Plasmid:V; (h) a nucleotide sequence encoding a biologically active fragment of a ACRP30-Like polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Plasmid:V; (i) a nucleotide sequence encoding an antigenic fragment of a ACRP30-Like polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Plasmid:V; and (j) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), or (i) above.

[0515] The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j) above, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence of the cDNA contained in Plasmid:V or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a polypeptide sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in Plasmid:V, the nucleotide sequence in SEQ ID NO:X encoding the polypeptide sequence as defined in column 10 of Table 1 or the complementary strand thereto, nucleotide sequences encoding the polypeptide as defined in column 10 of Table 1 or the complementary strand thereto, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides and nucleic acids.

[0516] In a preferred embodiment, the invention encompasses nucleic acid molecules which comprise, or alternatively, consist of a polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, under lower stringency conditions, to a polynucleotide in (a), (b), (c), (d), (e), (f), (g), (h), or (i), above, as are polypeptides encoded by these polynucleotides. In another preferred embodiment, polynucleotides

which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions, or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

[0517] In another embodiment, the invention provides a purified protein comprising, or alternatively consisting of, a polypeptide having an amino acid sequence selected from the group consisting of: (a) the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Plasmid:V; (b) the amino acid sequence of a mature form of a ACRP30-Like polypeptide having the amino acid sequence of SEQ ID NO:Y or the amino acid sequence encoded by the cDNA in Plasmid:V; (c) the amino acid sequence of a biologically active fragment of a ACRP30-Like polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Plasmid:V; and (d) the amino acid sequence of an antigenic fragment of a ACRP30-Like polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Plasmid:V.

[0518] The present invention is also directed to proteins which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, any of the amino acid sequences in (a), (b), (c), or (d), above, the amino acid sequence shown in SEQ ID NO:Y, the amino acid sequence encoded by the cDNA contained in Plasmid:V, the amino acid sequence as defined in column 10 of Table 1, an amino acid sequence encoded by the nucleotide sequence in SEQ ID NO:X, and an amino acid sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X. Fragments of these polypeptides are also provided (e.g., those fragments described herein). Further proteins encoded by polynucleotides which hybridize to the complement of the nucleic acid molecules encoding these amino acid sequences under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are the polynucleotides encoding these proteins.

[0519] By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100

nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence referred to in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

[0520] As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identiy are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the lenght of the subject nucleotide sequence, whichever is shorter.

[0521] If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the

purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

[0522] For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

[0523] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0524] As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence

referred to in Table 1 or a fragment thereof, the amino acid sequence encoded by the nucleotide sequence in SEQ ID NO:X or a fragment thereof, or to the amino acid sequence encoded by the cDNA in cDNA plasmid:V, or a fragment thereof, can be determined conventionally using known computer programs. A preferred method for determing the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237- 245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

[0525] If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

[0526] For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-

terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

[0527] The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which less than 50, less than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

[0528] Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

[0529] Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the

polypeptides of the present invention. For instance, as discussed herein, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988)).

[0530] Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

[0531] Furthermore, as discussed herein, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0532] Thus, the invention further includes polypeptide variants which show a functional activity (e.g. biological activity) of the polypeptide of the invention, of which they are a variant. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

[0533] The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, (e.g., encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion), irrespective of whether they encode a polypeptide having functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having functional activity include, inter alia, (1) isolating a gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting mRNA expression in specific tissues.

[0534] Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having functional activity of a polypeptide of the invention.

[0535] Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for example, the nucleic acid sequence of the cDNA in cDNA plasmid:V, the nucleic acid sequence referred to in Table 1 (SEQ ID NO:X), or fragments thereof, will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

[0536] For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

[0537] The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

[0538] The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989)). The resulting mutant molecules can then be tested for biological activity.

[0539] As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii)

substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification or (v) fusion of the polypeptide with another compound, such as albumin (including but not limited to recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

[0540] For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993)).

[0541] A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X, and/or the amino acid sequence encoded by the cDNA in cDNA plasmid:V which contains, in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature form and/or other fragments described herein), an amino acid sequence encoded by SEQ ID NO:X or fragments thereof, and/or the amino acid sequence encoded by cDNA plasmid:V or fragments thereof, is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative

amino acid substitutions are preferable. As discussed herein, any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, polypeptides of the present invention which are shown to be secreted can be used as targeting molecules once fused to other proteins.

[0542] Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

[0543] In certain preferred embodiments, proteins of the invention comprise fusion proteins wherein the polypeptides are N and/or C- terminal deletion mutants. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions mutants. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

[0544] Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

[0545] As one of skill in the art will appreciate, polypeptides of the present invention of the present invention and the epitope-bearing fragments thereof described above can be combined with heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with heterologous polypeptide sequences, for example, the polypeptides of the present invention may be fused with the constant domain of

immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988)). Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)).

Vectors, Host Cells, and Protein Production

[0546] The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

[0547] The polynucleotides of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[0548] The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[0549] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0550] Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

[0551] Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

[0552] A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

[0553] Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

[0554] In one embodiment, the yeast *Pichia pastoris* is used to express polypeptides of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O_2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O_2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. *See*, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J, *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

[0555] In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

[0556] Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

[0557] In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

[0558] In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and

Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0559] In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0560] The invention encompasses polypeptides of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

[0561] Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an

enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[0562] Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0563] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

[0564] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene

glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0565] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0566] The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

[0567] Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or an amino acid sequence encoded by SEQ ID NO:X or the complement of SEQ ID NO:X, and/or an amino acid sequence encoded by cDNA Plasmid:V (including fragments, variants, splice variants, and fusion proteins, corresponding to these as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of

the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

[0568] As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

[0569] Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, or contained in a polypeptide encoded by SEQ ID NO:X, and/or the cDNA plasmid:V). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are

between the heterologous sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0570] Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

[0571] Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

[0572] In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention

containing Flag® polypeptide seuqence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

[0573] The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[0574] Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate

recombinant polypeptides of the invention which contain a transmembrane domain (or hyrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Antibodies

[0575] Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0576] Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin

and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

[0577] The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

[0578] Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, or by size in contiguous amino acid residues. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

[0579] Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the

present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10^{-2} M, 10^{-2} M, 5 X 10^{-3} M, 10^{-3} M, 5 X 10^{-4} M, 10^{-4} M, 5 X 10^{-5} M, 10^{-5} M, 5 X 10^{-6} M, 10^{-6}M, 5 X 10^{-7} M, 10^{-7} M, 5 X 10^{-8} M, 10^{-8} M, 5 X 10^{-9} M, 10^{-9} M, 5 X 10^{-10} M, 10^{-10} M, 5 X 10^{-11} M, 10^{-11} M, 5 X 10^{-12} M, 10^{-12} M, 5 X 10^{-13} M, 10^{-13} M, 5 X 10^{-14} M, 10^{-14} M, 5 X 10^{-15} M, or 10^{-15} M.

[0580] The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[0581] Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferrably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at

least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

[0582] The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

[0583] Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

[0584] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[0585] The antibodies of the invention include derivatives that are modified, i.e, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0586] The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[0587] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0588] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0589] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

[0590] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be

produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0591] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0592] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

[0593] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries

derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0594] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and

Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0595] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

[0596] Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Polynucleotides Encoding Antibodies

[0597] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

[0598] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in

Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0599] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0600] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0601] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into

human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0602] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

[0603] Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

[0604] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0605] Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0606] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0607] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

[0608] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a

fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0609] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0610] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

[0611] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein

products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[0612] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0613] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance

to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[0614] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

[0615] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0616] Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method

known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

[0617] The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

[0618] The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions.

Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

[0619] As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

[0620] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag

provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

[0621] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc.

[0622] Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin,

etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0623] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon, ß-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol.,* 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0624] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0625] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In

Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

[0626] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0627] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

[0628] The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al., Cell, 96*:737-49 (1999)).

[0629] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-

Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

[0630] The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0631] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0632] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0633] ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0634] The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One

example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses

[0635] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0636] A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present

invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[0637] The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

[0638] The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0639] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Gene Therapy

[0640] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this

embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0641] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0642] For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[0643] In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

[0644] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

[0645] In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished

by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

[0646] In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

[0647] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[0648] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

[0649] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0650] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the

recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0651] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0652] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0653] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0654] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

[0655] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

[0656] The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

[0657] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably a polypeptide or antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0658] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0659] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be

administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0660] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0661] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

[0662] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also

Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[0663] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[0664] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0665] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate,

talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0666] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0667] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0668] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0669] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[0670] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

[0671] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression

of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

[0672] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0673] Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0674] One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically

binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0675] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

[0676] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0677] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0678] Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as

position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0679] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

[0680] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0681] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced

or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0682] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0683] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0684] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[0685] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0686] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Uses of the Polynucleotides

[0687] Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

[0688] The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome, thus each polynucleotide of the present invention can routinely be used as a chromosome marker using techniques known in the art.

[0689] Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably at least 15 bp (e.g., 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can optionally be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to SEQ ID NO:X will yield an amplified fragment.

[0690] Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

[0691] Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

[0692] For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

[0693] Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from the polynucleotide sequences in Table 1 and SEQ ID NO:X and (b) screening somatic cell hybrids containing individual chromosomes.

[0694] The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g. Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

[0695] Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library)). Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

[0696] Thus, once coinheritance is established, differences in a polynucleotide of the invention and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural

alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

[0697] Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using the polynucleotides of the invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

[0698] Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

[0699] In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the invention, where each probe has one strand containing a 31'mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

[0700] Where a diagnosis of a related disorder, including, for example, diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

[0701] By "measuring the expression level of polynucleotides of the invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the invention or the level of the mRNA encoding the polypeptide of the invention in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the related disorder or being determined by averaging levels from a population of individuals not having a related disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0702] By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains polypeptide of the present invention or the corresponding mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0703] The method(s) provided above may preferrably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the invention attached may be used to identify polymorphisms between the isolated polynucleotide sequences of the invention, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. Such a method is described in US Patents

5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

[0704] The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T.sub.m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

[0705] The present invention have uses which include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic

granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

[0706] Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Gelmann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelmann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelmann et al., supra)

[0707] For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580). However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness is not be limited to treatment of proliferative disorders of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

[0708] In addition to the foregoing, a polynucleotide of the present invention can be used to control gene expression through triple helix formation or through antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and

Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of polypeptide of the present invention antigens. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions.

[0709] Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

[0710] The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

[0711] The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying

and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

[0712] Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992)). Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

[0713] There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

[0714] The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays) or cell type(s) (e.g., immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention and/or cancerous and/or wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an

individual having such a disorder, relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

[0715] Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby an increase or decrease in the assayed gene expression level compared to the standard expression level is indicative of a disorder.

[0716] In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

[0717] Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

[0718] Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

[0719] Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon

(^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0720] In addition to assaying levels of polypeptide of the present invention in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

[0721] A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc, (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F, ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which express the polypeptide encoded by a polynucleotide of the invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0722] In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., polypeptides encoded by polynucleotides of the invention and/or antibodies) that are

associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0723] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

[0724] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0725] Techniques known in the art may be applied to label polypeptides of the invention (including antibodies). Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

[0726] Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a polypeptide of the present invention in cells or body

fluid of an individual; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0727] Moreover, polypeptides of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor supressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

[0728] Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described supra, and elsewhere herein). For example, administration of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

[0729] At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to

raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Diagnostic Assays

[0730] The compounds of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably humans. Such disorders include, but are not limited to, neural disorders (e.g., as described in "Neural Activity and Neurological Diseases" below), immune system disorders (e.g., as described in "Immune Activity" below), muscular disorders (e.g., as described in "Neural Activity and Neurological Diseases" below), reproductive disorders (e.g., as described in "Anti-Angiogenesis Activity" below), pulmonary disorders (e.g., as described in "Immune Activity" below), cardiovascular disorders (e.g., as described in "Cardiovascular Disorders" below), infectious diseases (e.g., as described in "Infectious Disease" below), proliferative disorders (e.g., as described in "Hyperproliferative Disorders", "Anti-Angiogenesis Activity" and "Diseases at the Cellular Level" below), cancerous diseases and conditions (e.g., as described in "Hyperproliferative Disorders", "Anti-Angiogenesis Activity" and "Diseases at the Cellular Level" below), and/or metabolic disorders (e.g., as described in "Endocrine Disorders" below).

[0731] ACRP30-Like proteins are believed to be involved in biological activities associated with glucose uptake, energy homeostasis, inflammation/immuneresponses, and angiogenesis/vascular repair. Accordingly, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, and/or treatment of diseases and/or disorders associated with aberrant ACRP30-Like activity.

[0732] In preferred embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, and/or treatment of Types I and II diabetes mellitus, obesity, and/or metabolic disorders described herein under "Endocrine Disorders". In additional embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, and/or treatment

of complications and disorders associated with Type I and II diabetes mellitus, including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations.

[0733] In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0734] In other embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, and/or treatment of diseases and/or disorders relating to immune disorders, endocrine disorders, and/or cardiovascular disorders as described under the sections entitled "Immune activity", "Endocrine Disorders", and "Cardiovascular Disorders" herein.

[0735] In certain embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose and/or prognose diseases and/or disorders disclosed in "Polynucleotides and Polypeptides of the Invention"; Table 4, column 3 (OMIM Reference(s)); as well as diseases and/or disorders associated with the tissue(s) in which the polypeptide of the invention is expressed, including the tissues disclosed in "Polynucleotides and Polypeptides of the Invention", and/or one, two, three, four, five, or more tissues disclosed in Table 3, column 2 (Library Code).

[0736] For a number of disorders, substantially altered (increased or decreased) levels of ACRP30-Like gene expression can be detected in tissues, cells or bodily fluids (e.g., sera, plasma, urine, semen, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" ACRP30-Like gene expression level, that is, the

ACRP30-Like expression level in tissues or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves measuring the expression level of the gene encoding the ACRP30-Like polypeptide in tissues, cells or body fluid from an individual and comparing the measured gene expression level with a standard ACRP30-Like gene expression level, whereby an increase or decrease in the gene expression level(s) compared to the standard is indicative of a ACRP30-Like disorder. These diagnostic assays may be performed *in vivo* or *in vitro*, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

[0737] The present invention is also useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed ACRP30-Like gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

[0738] By "assaying the expression level of the gene encoding the ACRP30-Like polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of the ACRP30-Like polypeptide or the level of the mRNA encoding the ACRP30-Like polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the ACRP30-Like polypeptide level or mRNA level in a second biological sample). Preferably, the ACRP30-Like polypeptide expression level or mRNA level in the first biological sample is measured or estimated and compared to a standard ACRP30-Like polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having the disorder. As will be appreciated in the art, once a standard ACRP30-Like polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0739] By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing ACRP30-Like polypeptides (including portions thereof) or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) and tissue sources found to express the full length or fragments thereof of a ACRP30-Like polypeptide. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0740] Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, Anal. Biochem. 162:156-159 (1987). Levels of mRNA encoding the ACRP30-Like polypeptides are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[0741] The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of ACRP30-Like polypeptides, in a biological sample (e.g., cells and tissues), including determination of normal and abnormal levels of polypeptides. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of ACRP30-Like polypeptides compared to normal control tissue samples may be used to detect the presence of tumors. Assay techniques that can be used to determine levels of a polypeptide, such as a ACRP30-Like polypeptide of the present invention in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Assaying ACRP30-Like polypeptide levels in a biological sample can occur using any art-known method.

[0742] Assaying ACRP30-Like polypeptide levels in a biological sample can occur using antibody-based techniques. For example, ACRP30-Like polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., *J. Cell Biol.*, 105:3087-3096 (1987)). Other antibody-based methods useful for detecting ACRP30-Like polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0743] The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the ACRP30-Like gene (such as, for example, cancer). The protein isolation methods employed herein may, for example, be such as those

described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the ACRP30-Like gene.

[0744] For example, antibodies, or fragments of antibodies, such as those described herein, may be used to quantitatively or qualitatively detect the presence of ACRP30-Like gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0745] In a preferred embodiment, antibodies, or fragments of antibodies directed to any one or all of the predicted epitope domains of the ACRP30-Like polypeptides may be used to quantitatively or qualitatively detect the presence of ACRP30-Like gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0746] In an additional preferred embodiment, antibodies, or fragments of antibodies directed to a conformational epitope of a ACRP30-Like polypeptide may be used to quantitatively or qualitatively detect the presence of ACRP30-Like gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0747] The antibodies (or fragments thereof), and/or ACRP30-Like polypeptides of the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of ACRP30-Like gene products or conserved variants or peptide fragments thereof. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or ACRP30-Like polypeptide of the present invention. The antibody (or fragment thereof) or ACRP30-Like polypeptide is

preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the ACRP30-Like gene product, or conserved variants or peptide fragments, or ACRP30-Like polypeptide binding, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

[0748] Immunoassays and non-immunoassays for ACRP30-Like gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of binding ACRP30-Like gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

[0749] The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled anti-ACRP30-Like polypeptide antibody or detectable ACRP30-Like polypeptide. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

[0750] By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many

other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0751] The binding activity of a given lot of anti-ACRP30-Like polypeptide antibody or ACRP30-Like antigen polypeptide may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0752] In addition to assaying ACRP30-Like polypeptide levels or polynucleotide levels in a biological sample obtained from an individual, ACRP30-Like polypeptide or polynucleotide can also be detected *in vivo* by imaging. For example, in one embodiment of the invention, ACRP30-Like polypeptide and/or anti-ACRP30-Like antigen antibodies are used to image diseased cells, such as neoplasms. In another embodiment, ACRP30-Like polynucleotides of the invention (e.g., polynucleotides complementary to all or a portion of a particular ACRP30-Like mRNA transcript) and/or anti-ACRP30-Like antibodies (e.g., antibodies directed to any one or a combination of the epitopes of a ACRP30-Like polypeptide of the invention, antibodies directed to a conformational epitope of a ACRP30-Like polypeptide of the invention, or antibodies directed to the full length polypeptide expressed on the cell surface of a mammalian cell) are used to image diseased or neoplastic cells.

[0753] Antibody labels or markers for *in vivo* imaging of ACRP30-Like polypeptides include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. Where *in vivo* imaging is used to detect enhanced levels of ACRP30-Like polypeptides for diagnosis in humans, it may be preferable to use human antibodies or "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using techniques described herein or otherwise known in the art. For example methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science 229*:1202 (1985); Oi et al., *BioTechniques 4*:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne *et al., Nature 312*:643 (1984); Neuberger *et al.,*

Nature 314:268 (1985).

[0754] Additionally, any ACRP30-Like polypeptides whose presence can be detected, can be administered. For example, ACRP30-Like polypeptides labeled with a radio-opaque or other appropriate compound can be administered and visualized *in vivo*, as discussed, above for labeled antibodies. Further such ACRP30-Like polypeptides can be utilized for *in vitro* diagnostic procedures.

[0755] A ACRP30-Like polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain ACRP30-Like protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0756] With respect to antibodies, one of the ways in which the anti-ACRP30-Like polypeptide antibody can be detectably labeled is by linking the same to a reporter enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL,; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The reporter enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Reporter enzymes which can be used to detectably label the antibody include, but are not limited to, malate

dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the reporter enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0757] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect ACRP30-Like polypeptides through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[0758] It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[0759] The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0760] The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0761] Likewise, a bioluminescent compound may be used to label the antibody of the

present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Methods for Detecting Diseases

[0762] In general, a disease may be detected in a patient based on the presence of one or more ACRP30-Like proteins of the invention and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, urine, and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a disease or disorder, including cancer and/or as described elsewhere herein. In addition, such proteins may be useful for the detection of other diseases and cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding ACRP30-Like polypeptides, which is also indicative of the presence or absence of a disease or disorder, including cancer. In general, ACRP30-Like polypeptides should be present at a level that is at least three fold higher in diseased tissue than in normal tissue.

[0763] There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. See, e.g., Harlow and Lane, *supra.* In general, the presence or absence of a disease in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

[0764] In a preferred embodiment, the assay involves the use of a binding agent(s) immobilized on a solid support to bind to and remove the ACRP30-Like polypeptide of the invention from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein

G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include ACRP30-Like polypeptides and portions thereof, or antibodies, to which the binding agent binds, as described above.

[0765] The solid support may be any material known to those of skill in the art to which ACRP30-Like polypeptides of the invention may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for the suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 ug, and preferably about 100 ng to about 1 ug, is sufficient to immobilize an adequate amount of binding agent.

[0766] Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group

on the support with an amine and an active hydrogen on the binding partner (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

Gene Therapy Methods

[0767] Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

[0768] Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the present invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide of the present invention. Such methods are well-known in the art. For example, see Belldegrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112 (1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

[0769] As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[0770] In one embodiment, the polynucleotide of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotide of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

[0771] The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

[0772] Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotide of the present invention.

[0773] Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[0774] The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach,

intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

[0775] For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

[0776] The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[0777] The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

[0778] The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

[0779] In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

[0780] Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

[0781] Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

[0782] Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can

also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

[0783] For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

[0784] The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+}-EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta (1975) 394:483; Wilson et al., Cell (1979) 17:77); ether injection (Deamer, D. and Bangham, A., Biochim. Biophys. Acta (1976) 443:629; Ostro et al.,

Biochem. Biophys. Res. Commun. (1977) 76:836; Fraley et al., Proc. Natl. Acad. Sci. USA (1979) 76:3348); detergent dialysis (Enoch, H. and Strittmatter, P., Proc. Natl. Acad. Sci. USA (1979) 76:145); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem. (1980) 255:10431; Szoka, F. and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA (1978) 75:145; Schaefer-Ridder et al., Science (1982) 215:166), which are herein incorporated by reference.

[0785] Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

[0786] U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

[0787] In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding a polypeptide of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

[0788] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and

$CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[0789] The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding a polypeptide of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express a polypeptide of the present invention.

[0790] In certain other embodiments, cells are engineered, ex vivo or in vivo, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses a polypeptide of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis.109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

[0791] Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

[0792] Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or

packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

[0793] In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

[0794] For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either ex vivo or in vivo. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a polypeptide of the invention.

[0795] Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4,

1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

[0796] Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

[0797] The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

[0798] The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

[0799] The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

[0800] Preferably, the polynucleotide encoding a polypeptide of the present invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or

heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

[0801] Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

[0802] A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

[0803] Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

[0804] Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

[0805] Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier

capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

[0806] Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

[0807] Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

Biological Activities

[0808] Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention, do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides, and agonists or antagonists could be used to treat the associated disease.

[0809] ACRP30-Like proteins are believed to be involved in biological activities associated with glucose uptake, energy homeostasis, inflammation/immune responses, and angiogenesis/vascular repair. Accordingly, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, and/or treatment of diseases and/or disorders associated with aberrant ACRP30-Like activity.

[0810] In preferred embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, and/or treatment of Types I

and II diabetes mellitus, obesity, and/or metabolic disorders described herein under "Endocrine Disorders". In additional embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, and/or treatment of complications and disorders associated with Type I and II diabetes mellitus, including, but not limited to, seizures, mental confusion, drowsiness, nonketotic hyperglycemic-hyperosmolar coma, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section below), dyslipidemia, kidney disease (e.g., renal failure, nephropathy and/or as described in the "Renal Disorders" section below), endocrine disorders (as described in the "Endocrine Disorders" section below), obesity, nerve damage, neuropathy, impotence, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section below, especially of the urinary tract and skin), carpal tunnel syndrome, Dupuytren's contracture, and amputations.

[0811] In additional preferred embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to regulate weight gain, weight loss, and/or obesity.

[0812] In other embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention, and/or treatment of diseases and/or disorders relating to immune disorders, endocrine disorders, and/or cardiovascular disorders as described under the sections entitled "Immune activity" , "Endocrine Disorders",and "Cardiovascular Disorders" herein.

[0813] In certain embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose and/or prognose diseases and/or disorders disclosed in "Polynucleotides and Polypeptides of the Invention"; Table 4, column 3 (OMIM Reference(s)); as well as diseases and/or disorders associated with the tissue(s) in which the polypeptide of the invention is expressed, including the tissues disclosed in "Polynucleotides and Polypeptides of the Invention", and/or one, two, three, four, five, or more tissues disclosed in Table 3, column 2 (Library Code).

[0814] More generally, polynucleotides, translation products and antibodies corresponding to this gene may be useful for the diagnosis, detection and/or treatment of diseases and/or disorders associated with the following systems.

Immune Activity

[0815] Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

[0816] In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an immune response generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 3, column 2 (Library Code).

[0817] Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing, and/or prognosing immunodeficiencies, including both congenital and acquired immunodeficiencies. Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including congenital and acquired

agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type), Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID), common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.

[0818] In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are treated, prevented, diagnosed, and/or prognosing using the polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof.

[0819] Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

[0820] In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

[0821] Other immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, include, but are not limited to, chronic granulomatous disease, Chédiak-Higashi syndrome, myeloperoxidase deficiency, leukocyte glucose-6-phosphate dehydrogenase deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic alymphoplasia-aplasia,

immunodeficiency with thymoma, severe congenital leukopenia, dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

[0822] In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are treated, prevented, diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

[0823] In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

[0824] The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

[0825] Autoimmune diseases or disorders that may be treated, prevented, diagnosed and/or prognosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic anemia, thrombocytopenia, autoimmune thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henloch-Scoenlein purpura), autoimmunocytopenia, Goodpasture's syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.